

Dosage Effect at the Sugary-2 Locus on Maize Starch Structure and Function¹

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ABSTRACT

Cereal Chem. 71(5):464-468

Structural characteristics and functional properties of starches isolated from kernels of a sugary-2 (*su*₂) dosage series were examined to determine whether the normal allele (*Su*₂) was completely dominant to the recessive allele (*su*₂). Differential scanning calorimetry revealed intermediate values for gelatinization onset, gelatinization peak, range, total enthalpy, and retrogradation among genotypes possessing one and two mutant *su*₂ alleles. No effect of gene dosage on amylose content was observed, but X-ray diffraction patterns revealed an intermediate degree of crystallinity relative

to normal and mutant genotypes upon addition of two *su*₂ alleles. Development of a peak at 19°2θ became more evident upon increasing doses of the *su*₂ allele. Viscosity of the starch paste and gel strength resulting from the genotype possessing two doses of the *su*₂ allele exceeded that of both mutant and normal genotypes. An intermediate retrogradation as detected by differential scanning calorimetry and from gel strengths of starches after storage for seven days suggested an increased stability of the *Su*₂*su*₂*su*₂ starch relative to that of normal starch.

The recessive sugary-2 (*su*₂) allele of maize (*Zea mays* L.) was identified by Eyster (1934) and found to reside on chromosome six of maize. Until now, it has not been associated with any genetic lesion. Characteristics distinguishing *su*₂ from the normal (nonmutant) genotype include reduced starch content, a 10–15% increase in amylose content, and a lowered birefringence end-point temperature, reflecting its low gelatinization temperature (Pfahler et al 1957, Kramer et al 1958). More recently, Inouchi et al (1984) found gelatinization endotherms from differential scanning calorimetry (DSC) to have lower onset (*T*_o) and peak (*T*_p) temperatures and a reduced enthalpy (ΔH) when compared with normal corn starch. They also described starch of *su*₂ as having an A-type X-ray diffraction pattern. However, the diffraction peaks were broad and weak, reflecting a lower degree of crystallinity than that of normal starch. Compared with other maize genotypes, a high susceptibility of *su*₂ starch to digestion by pancreatic α -amylase was demonstrated by Sandstedt et al (1962). It was suggested that starch of *su*₂ may differ from normal starch because of differences in the bonding of starch molecules or anomalous linkages within the molecules. Little difference in the fine structure of amylopectin from *su*₂ was found as compared with normal starch on the basis of unit chain length distributions (Inouchi et al 1987). Takeda and Preiss (1993), however, found that, when compared with that of normal starch, the amylopectin of *su*₂ starch was composed of larger sized long B-chains, which were more poorly branched, leading to increased iodine affinity value.

Interest in the *su*₂ mutant emerged when starch isolated from genotypes containing this allele in combination with dull (*du*) and sugary-1 (*su*₁) was found to contain about 77% amylose (Dvovich et al 1951, Dunn et al 1953). Improved nutritional quality of *su*₂ also was reported as a result of its high susceptibility to α -amylase digestion. This led Sandstedt et al (1962) to suggest its use in improving feed value. Enhanced grain quality was later recognized by Glover et al (1975) because the *su*₂ allele in combination with opaque-2 (*O*₂) resulted in kernel density nearly equal to that of ordinary dents. More recently, several novel starches

resulting from *su*₂ alone and in combination with other alleles such as *du*, amylose-extender (*ae*), and waxy (*wx*) have produced starches with properties resembling those of some modified starches (Friedman et al 1988a,b; White et al 1993).

The lack of complete dominance of normal alleles at the mutant loci *ae* and *wx* on amylose content has been well documented (for review see Shannon and Garwood 1984). Similar studies have shown that gene dosage effects of *su*₂ on amylose content do not exist, with the exception of its presence in a *su*₁ background (Kramer and Whistler 1949). Studies considering only amylose content, however, may fail to reveal dosage effects on other starch characteristics. For example, Boyer et al (1980) found that, although increasing doses of *ae* in a *wx* background did not increase amylose content, average chain length did increase. Also, Yamada et al (1978) found that, upon addition of the *ae* allele in a *wx* background, gelatinization temperature increased.

Sanders et al (1990) emphasized the need for novel starches from mutant genotypes having the desired properties of chemically modified starches in order to avoid problems associated with regulatory approval for food use. Characterization of starches of lesser-used endosperm mutants, intermediate in expression, may reveal starch types with unique properties. The objective of this study was to investigate possible gene dosage effects at the *su*₂ locus and determine whether novel starch properties exist among the dosage intermediates.

MATERIALS AND METHODS

Material

In 1992, self-pollinations and reciprocal crosses were made among stocks of Oh43 and Oh43 *su*₂*su*₂ in a nursery near Ames, IA. Mature ears were harvested and dried at 38°C for five days to ~13% moisture content. Samples were stored in a cold room at 4°C and 45% rh until analyzed.

Starch Isolation

A small-scale starch isolation procedure was used for the bulk of five kernels obtained from the center portion of the ear as described by White et al (1990). Ten separate extractions were made, each from a separate ear, from each genotype for DSC and colorimetric determination of amylose (AM) content. For all other analyses (iodine affinity, X-ray diffraction, amylography, and gel strength), starch was isolated from a single large-scale isolation by pooling the remaining grain of the same 10 ears. The large-scale starch isolation procedure used was that described by Steinke and Johnson (1991). After starch isolation, samples were purified with 5 volumes of 0.2M sodium chloride-toluene (5:1, v/v) at least five times, and starch granules were allowed to settle. The final sediment was washed three times with distilled water and dried at 45°C for 24 hr. Because of the low gelatinization

¹This is a joint contribution of Journal Paper J-15594 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, and Field Crops Research Unit, USDA-ARS, Projects 3128 and 3082.

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temperature of $su_2su_2su_2$ starch, microscopic examination of the starches was conducted to verify that gelatinization did not result from the elevated temperature achieved during steeping or drying. Similar intensities of birefringence reported by Brown (1971) for normal and $su_2su_2su_2$ starch were observed from these samples. In addition, the lack of swollen granules provided evidence that no gelatinization occurred during the starch-isolation procedure.

Amylose Determination

AM content was determined colorimetrically by dissolving ~5.0 mg of starch in 10 ml of 90% dimethyl sulfoxide containing 6×10^{-3} iodine (Knutson 1986). One milliliter of the dissolved sample was diluted to 9 ml with H_2O , and the absorbance was measured at 600 nm on a spectrophotometer (Hitachi U-2000, Tokyo, Japan). Purified AM was prepared from maize starch as described by Schoch (1942) and used to construct a standard curve. Iodine affinity (IA) was determined by amperometric titration from starches of each genotype as described by Schoch (1964). These starches were defatted by refluxing 24 hr in 85% methanol. Values for IA were expressed as milligrams of iodine bound to 100 mg of starch.

Differential Scanning Calorimetry

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). Approximately 4.0 mg (dwb) of starch was weighed into aluminum sample pans and 8 mg of distilled water was added. Samples were heated from 30 to 102°C at a rate of 10°C/min. DSC parameters for this study included enthalpy (ΔH), gelatinization onset temperature (T_o), peak temperature (T_p), and range. The parameters T_o , T_p , and ΔH were given directly by the DSC software. Range was calculated by $2(T_p - T_o)$ according to Krueger et al (1987). Samples were stored for seven days at 4°C and rerun to determine starch gel retrogradation (%R) as described by White et al (1989).

Starch Granule Size

Isolated starch granules were dispersed in ethanol and mounted on slides. The preparations were placed on a Laborlux light microscope fitted with a television camera. The microscope system was attached to a digitizing Colorado video unit linked to a Kevex Delta IV (San Carlos, CA) computer with an image analysis software program. Fields of starch granules from different samples were viewed with a 40× objective and then digitized and processed for image analysis. Data collected were expressed in Waddell diameter and averaged from at least 200 granules per genotype.

X-Ray Diffractometry

X-ray diffraction patterns were collected with a Siemens (Madison, WI) D-500 X-ray diffractometer equipped with a copper X-ray tube and graphite monochromator. Samples containing 10% moisture were scanned from 4 to 40°2θ. The degree of crystallinity was determined from a single diffraction pattern by using the method of Koksel et al (1993). Relative crystallinity (RC%) was calculated as the ratio of the upper crystalline area to the lower amorphous area of the diffraction pattern. The total area was calculated as the sum total of the crystalline and amorphous areas.

Amylography

A Brabender Viscoamylograph (C.W. Brabender Instruments, S. Hackensack, NJ) equipped with a 700-cmg sensitivity cartridge operating at a bowl speed of 75 rpm was used to determine pasting characteristics of starch suspensions (8% w/w, dwb) adjusted to a pH of 5.5. The temperature was raised from 30 to 95°C at a rate of 1.5°C/min and maintained at 95°C for 30 min, then lowered to 50°C at the same rate and held for 30 min. Measurements for each genotype were made in duplicate.

Gel Strength

Starch pastes from each genotype prepared by using the Brabender Viscoamylograph were used to measure gel strength after storage

for one and seven days at 4°C. Starch paste was poured into aluminum dishes (27 mm i.d. × 27 mm), taped around the rims to increase the depth, and later cut back to produce a fresh surface before analysis. From each of two replicates, gel strength was measured at five different locations on each gel sample (five measurements per dish = total of four dishes) by using a Voland texture analyzer (Texture Technologies, Scardale, NY) as previously described by Takahashi et al (1989).

RESULTS AND DISCUSSION

AM Content

Results obtained from the two methods used for the determination of AM are shown in Table I. The AM% in starch of $su_2su_2su_2$ was ~10% greater than that in starch of normal corn. These values correspond to the larger IA resulting from the mutant genotype. Starch from su_2 dosage intermediate genotypes ($Su_2Su_2su_2$ and $Su_2su_2su_2$) did not differ from the normal parent with respect to AM for either method of determination. The data are in agreement with previous studies, which concluded that gene dosage has no effect on amylose content.

Thermal Properties

Representative DSC thermograms of starch gelatinized from each genotype are shown in Figure 1. DSC parameter means of thermograms determined from 10 ears per genotype are given in Table II. Thermal properties, including T_o , T_p , and ΔH from starch of the homozygous recessive su_2 genotype, were lower than those of normal starch, which is in agreement with results obtained by Inouchi et al (1991). The lowered T_o corresponds to the observed loss of birefringence occurring at a lower temperature in su_2 compared with that of normal starch (Pfahler et al 1957, Kramer et al 1958). Brown et al (1971) suggested that the lower gelatinization temperature caused by the su_2 allele may result from a reduction in the association between starch molecules within the granule. Rerunning the samples after storage at 4°C revealed that the %R of $su_2su_2su_2$ starch was substantially less than that of normal corn starch. The low %R reflects a possible increase in stability of the starch gel as discussed by White et al (1989).

In contrast to the AM%, a dosage effect at the su_2 locus was present for T_o , T_p , range, and ΔH . A single dose of su_2 significantly reduced T_o and T_p , although the differences were not large and more closely resembled values from normal maize starch. Two doses of the su_2 allele resulted in T_o and T_p nearly intermediate to that of the normal and mutant genotypes. Also, the range increased to a value similar to that of the mutant, and ΔH was reduced relative to the normal genotype. The %R of the sample also decreased upon each addition of the mutant allele, indicating a possible increase in gel stability. Clearly, characteristics of the mutant genotype governing thermal properties were retained in the dosage intermediates, and these characteristics did not seem to be a result of increased AM.

TABLE I
Amylose Content (AM) and Average Granule Diameters of Starches from the Sugary-2 Dosage Series in the Maize Inbred Oh43

Genotype	AM, % ^a	Iodine Affinity ^b (g/100 g of starch)	Average Granule Diameter, μm
$Su_2Su_2Su_2$	25.1	5.0	5.7 ± 1.5 ^c
$Su_2Su_2su_2$	24.7	4.9	6.0 ± 1.7
$Su_2su_2su_2$	24.7	5.0	5.4 ± 2.0
$su_2su_2su_2$	39.3	8.1	5.6 ± 1.5
LSD ^d	1.5		

^a Mean of 10 ears per genotype.

^b Mean of two determinations.

^c Standard deviation.

^d Least significant difference ($P \leq 0.05$).

Starch Granule Size

Means for average granule diameters for genotypes are shown in Table I. Mean granule diameters were small compared to those previously reported for normal and su_2 starch (Brown et al 1971). These differences may have been the result of the method chosen for analysis, of growing conditions, or the effect of genetic background. No substantial difference in mean diameter was seen among genotypes from the su_2 dosage series. The results are in agreement with previous studies, indicating that starch granule sizes of the su_2 mutant were similar to those of normal (Brown et al 1971). Differences in thermal properties of dosage intermediates compared to normal starch, therefore, did not seem to be related to size of granules.

X-Ray Diffraction

An A-type X-ray diffraction pattern was observed for all four genotypes (Fig. 2). Previous studies (Dvornik et al 1951, Inouchi et al 1984) also indicated an A-type pattern for starch of su_2 , with the exception of a study by Creech (1968) in which a B-type pattern was described. Diffraction peaks of $su_2su_2su_2$ starch were

more broad and weaker than were peaks of normal starch as a result of the decreased crystallinity of the starch. Inouchi et al (1984) noted that, because crystallinity is primarily caused by the amylopectin fraction, $su_2su_2su_2$ starch should be expected to have a lesser degree of crystallinity than that of normal starch. Genotypes with AM contents similar to each other, such as normal and the dosage intermediates, would be expected to have relatively similar crystallinities. X-ray diffraction patterns of dosage intermediates also revealed an A-type diffraction pattern resembling that of normal corn starch. RC% values among the genotypes shown in Table III suggest a dosage effect in the presence of two su_2 alleles where %RC was reduced from 46% seen in normal corn to 40%. Close examination of diffraction patterns suggest a slight increase in the peak at $19^\circ 2\theta$ upon increasing doses of the su_2 allele. This increase may be a result of the diminishing peak at $17^\circ 2\theta$ allowing better resolution of the peak. Results of this study indicate that a structural component of the starch other than amylopectin may be involved in influencing relative crystallinity of the starch.

Pasting Properties

Values describing significant points of the amylogram are shown in Table IV. Starch of the $su_2su_2su_2$ mutant clearly differed from

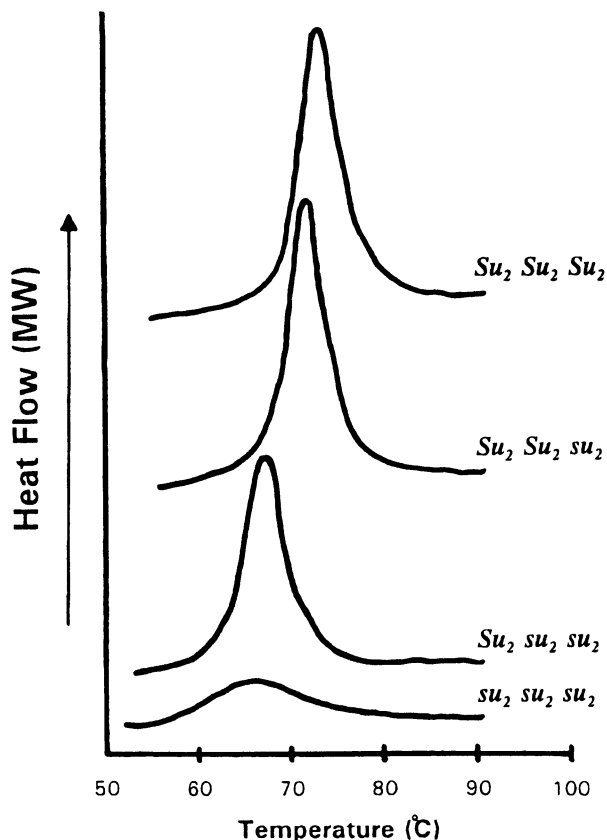


Fig. 1. Representative differential scanning calorimetry thermograms from starch gelatinization of the sugary-2 (su_2) dosage series in the maize inbred Oh43.

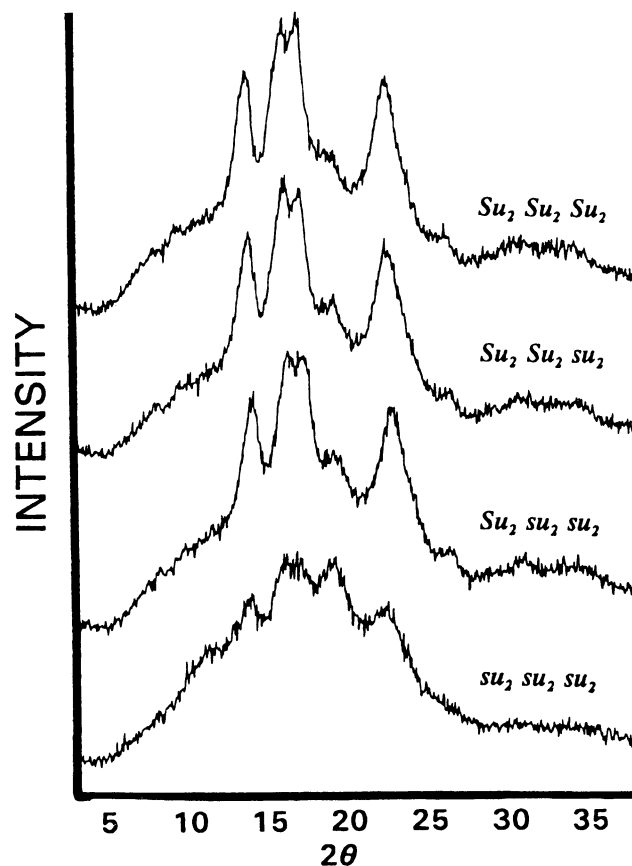


Fig. 2. X-ray diffractograms of starch from the sugary-2 (su_2) dosage series in the maize inbred Oh43.

TABLE II
Differential Scanning Calorimetry Parameter Means of Starches
from the Sugary-2 Dosage Series in the Maize Inbred Oh43

Genotype	T_{0s} , °C ^a	T_p , °C ^b	Range, °C	ΔH , cal/g ^c	%R ^d
$Su_2Su_2Su_2$	67.3	71.0	7.4	2.9	58.8
$Su_2Su_2su_2$	65.9	69.6	7.4	2.8	54.5
$Su_2su_2su_2$	62.3	66.9	9.3	2.5	52.4
$su_2su_2su_2$	58.3	63.0	9.4	0.9	43.9
LSD ^e	0.8	0.5	1.0	0.1	3.6

^aGelatinization onset.

^bGelatinization peak.

^cEnthalpy of gelatinization.

^dPercent retrogradation; based on ΔH retrogradation/ ΔH gelatinization.

^eLeast significant difference ($P \leq 0.05$).

TABLE III
Relative Crystallinity of X-Ray Diffractograms from Starch
of the Sugary-2 Dosage Series in the Maize Inbred Oh43

Genotype	RC% ^a	Total Area ^b (cm ²)
$Su_2Su_2Su_2$	46.3	59.1
$Su_2Su_2su_2$	46.4	56.4
$Su_2su_2su_2$	40.4	59.8
$su_2su_2su_2$	21.6	56.8

^aRelative crystallinity = upper crystalline area/lower amorphous area.

^bSum total of crystalline and amorphous areas.

TABLE IV
Pasting Properties Determined from Brabender Viscoamylograph of Starches from the Sugary-2 Dosage Series in the Maize Inbred Oh43^a

Genotype	Pasting Onset (°C)	Peak Temperature (°C)	Brabender Viscosity Units					
			Peak Viscosity	95°C	95°C Hold	50°C	Setback ^b	50°C Hold
<i>Su₂Su₂Su₂</i>	70	88.8	870	765	575	1,240	670	1,210
<i>Su₂Su₂Su₂</i>	68	89.8	870	810	620	1,360	740	1,415
<i>Su₂Su₂Su₂</i>	70	94.2	820	820	700	1,660	960	1,630
<i>su₂su₂su₂</i>	92	... ^c	...	20	60	320	260	260
LSD ^d	2.6	2.2	72	43	17	53	36	33

^aStarch concentration of 8% (w/w, dwb). Values are means of two determinations.

^bSetback denotes the difference between viscosity after a 30-min hold at 95°C and immediately after cooling to 50°C.

^cNo peak observed.

^dLeast significant difference ($P \leq 0.05$).

TABLE V
Gel Strength of Starches from the Sugary-2 Dosage Series in the Maize Inbred Oh43^a

Genotype	Firmness, g-force			Stickiness, g-force		
	D1 ^b	D7 ^c	D7/D1	D1	D7	D7/D1
<i>Su₂Su₂Su₂</i>	42	62	1.5	-14	-38	2.7
<i>Su₂Su₂Su₂</i>	45	55	1.2	-16	-25	1.6
<i>Su₂Su₂Su₂</i>	49	57	1.2	-15	-24	1.6
<i>su₂su₂su₂</i>	23	30	1.3	-17	-23	1.4
LSD ^d	4	10		1	17	

^aValues are the average of two separate samples (five determinations per sample) from each of two replicates.

^bSamples analyzed after one day of storage at 4.0°C.

^cSamples analyzed after seven days of storage at 4.0°C.

^dLeast significant difference ($P \leq 0.05$).

that of normal corn starch by having a delayed pasting onset, no initial viscosity peak, and a low final viscosity after cooling. The pasting pattern of the *su₂su₂su₂* starch resembled that of other starch types, such as *du*, which was reported by Wang et al (1992) to have a higher pasting onset temperature and a lower viscosity than that of normal starch. The relatively high AM content of *du₁* was thought to cause the higher pasting temperature resulting from the increased bonding of amylose molecules within the granule. A more loosely branched amylopectin structure of *du ae* relative to other high-amylose starches was suggested by Katz (1991) to result in a pasting property similar to that of *du* and *su₂*. The *su₂* starch also may have a loosely branched amylopectin structure, thus accounting for its unusual pasting properties. This idea is in agreement with observations of Brown et al (1971) describing a reduced association of molecules in the granules of *su₂*. Effects of gene dosage on pasting properties were observed (Table IV). An increased peak temperature occurred with each addition of the *su₂* allele, and two doses significantly ($P < 0.05$) increased the temperature from 88.8 to 94.2°C. Pasting onset temperature measured for values exceeding 10 BU did not differ greatly among dosage intermediates and normal starch, but starch possessing two alleles of *su₂* lagged in viscosity after pasting onset when compared with normal starch. Pasting onset temperature of the genotype possessing two *su₂* alleles, however, was intermediate relative to the normal and mutant genotype when based on values exceeding 100 BU. Viscosities of pastes from dosage intermediates upon heating to 95°C and until completion of the cooling cycle exceeded those of both normal and mutant genotypes. A structural basis for the increased viscosity of the dosage intermediates relative to normal starch is unclear. Characteristics of the fully recessive *su₂* starch (other than AM content) are likely to be retained in the intermediates and to play a role in starch structure. Structural changes leading to the reduced relative crystallinity in the *Su₂su₂su₂* genotype are examples. The double mutant combination of *ae wx* described by Yamada et al (1978) also possessed pasting properties similar to that of the *Su₂su₂su₂* genotype with respect to the higher pasting onset temperature; it maintained a more viscous paste than did both normal and

waxy. Structurally, *ae wx* starch differs from waxy and normal starches in having longer mean chain lengths. Further studies may reveal unit chain length differences between normal starch and starches of the *su₂* dosage intermediate genotypes.

Gel Strength

Table V shows gel strengths of starch pastes prepared in the Brabender Viscoamylograph after storage for one and seven days. Gels from normal starch were almost twice as firm as those from *su₂su₂su₂* starch after one day of storage. Also, the addition of *su₂* alleles increased starch gel strength to values greater than that of normal starch. These results corresponded to final viscosities of starch pastes as determined by amylography. Although, no structural basis for the differences in gel strength is clear, a more concentrated soluble phase or a more linear amylose of dosage intermediates may result in the increases (Takahashi and Seib 1988). After seven days of storage, gel strength increased for all genotypes. Gel strength of *su₂su₂su₂* samples remained weakest; no significant differences were seen among the normal and dosage intermediate genotypes. Interestingly, the ratio of firmness of gels between one and seven days of storage was lower for all genotypes containing at least one *su₂* allele. Because retrogradation of starch gels is associated with syneresis of water, leading to denser gels, the change in firmness may reflect stability of gels under refrigerator storage, with the greater the relative increase in firmness during storage, the greater the tendency to retrograde. These findings agree with the DSC results in which there was a decrease in degree of retrogradation of starches with each addition of a *su₂* allele.

Gel stickiness after one day of storage revealed that the value for *su₂su₂su₂* was greater than that of normal starch, whereas genotypes containing either one or two *su₂* alleles were intermediate. After seven days, however, no significant differences were seen. A continuous decrease in the ratios of values determined after one and seven days was seen upon each addition of the *su₂* allele.

CONCLUSIONS

The *su₂* mutant of maize was completely recessive to the normal allele with respect to AM content, thus confirming previous findings. Thermal properties measured by DSC clearly revealed a dosage effect for all parameters. Certain structural characteristics of starch from the *su₂su₂su₂* genotype seemed to be retained in the dosage intermediates, because relative crystallinity decreased in starch of the *Su₂su₂su₂* genotype. Functional properties of starches from the *Su₂su₂su₂* genotype also differed from those of normal and mutant genotypes because pasting onset was delayed, and final viscosity was higher than in normal starch. Gel strengths also exceeded that of the normal genotypes.

This study has shown that novel starch types can be achieved through development of dosage intermediate genotypes. Commercial production of starch from dosage intermediates, however, would not be practical because of the segregating nature of kernels in hybrid corn. Starch from hybrid *su₂su₂su₂* genotypes with traits of the dosage intermediates may be possible through the use of

modifying genes or the development of additional alleles at the *su*₂ locus (Curme 1955, Vineyard et al 1958).

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[Received December 8, 1993. Accepted May 30, 1994.]