Physicochemical Properties of Starches from Mutant Genotypes of the Oh43 Inbred Line¹

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ABSTRACT

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The physicochemical properties of 17 mutant genotypes of the Oh43 inbred line were investigated to clarify the relationship between structural characteristics and physicochemical properties and among the properties themselves. These physicochemical properties included blue value (BV), maximum absorbance wavelength (λ_{max}) , limiting viscosity number ([η]), swelling power and solubility at 85°C, and percent light transmittance (%T) of starch paste at 650 nm. Pasting properties were determined by means of Brabender viscoamylography, gel strength by texture analysis, and thermal properties by differential scanning calorimetry. Amylose content was the most important structural characteristic affecting the physicochemical properties of starch. Amylose content was significantly (P < 0.01) correlated with BV (r = 0.96) and λ_{max} (r = 0.81) and was negatively correlated with $[\eta]$ (r = -0.83), %T(r = -0.88), swelling power (r = -0.86), and peak viscosity (r = -0.97). Other structural characteristics, including intermediate material content, average chain length of debranched amylopectin, and ratio of long B chains to short B chains plus A chains of amylopectin, were weakly correlated with properties. Some significant correlations were found among properties, including BV, %T, swelling power, and peak viscosity.

Starch, the most important reserve carbohydrate in the plant world, is used widely in food processing and other industries. A growing interest in using native starches in the modern food industry has created a demand for new alternative sources of

Maize is unique among higher plants in its number of genetically accessible mutants and in the degree to which it has been characterized. Much work has helped determine starch structures of maize mutants (Boyer et al 1976; Ikawa et al 1978, 1981; Yeh et al 1981; Inouchi et al 1983, 1987; Boyer and Liu 1985; Fuwa et al 1987), yet little emphasis has been focused on the physicochemical properties of these mutants. In general, blue value (BV), iodine absorption spectrum (λ_{max}), and β -amylolysis limit are used to distinguish amylopectin from amylose in mutant starches (Boyer et al 1976, Yeh et al 1981, Fuwa et al 1987). No other physicochemical properties, other than limiting viscosity number $([\eta])$, have been studied to characterize the starches of maize mutants.

In a previous study (Wang et al 1993), 17 maize mutant genotypes of the Oh43 inbred line were characterized for their starch fine structures. The objectives of the present study were to examine the physicochemical properties of starches from these 17 maize mutant genotypes and to clarify the relationships between these properties and starch structures.

MATERIALS AND METHODS

Materials

Mature kernels of Oh43 inbred and its single and double mutants (Table I) were harvested from a summer nursery near Ames, IA, in 1991. Development of the genotypes and sampling and storing of the kernels were described previously (Wang et al 1992).

Starches were isolated by a wet-milling procedure (Steinke and Johnson 1991). The isolated starches were purified by treating with 5 volumes of 0.2M sodium chloride-toluene (5:1, v/v) at least five times. After each treatment, the starch granules were sedimented by centrifugation. The final sediment was washed three times with distilled water and dried at 45°C for 24 hr. All the starches used in this study were obtained from a single isolation

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Blue Value and Iodine-Absorption Spectrum

The BV was determined according to Gilbert and Spragg (1964), and the same sample was used to measure λ_{max} (700-500 nm). Two separate determinations were done on each starch genotype.

Limiting Viscosity Number

Determination of $[\eta]$ (milliliters per gram) was made with an Ostwald viscometer (Fisher Scientific, Pittsburgh, PA) at 22.5°C according to the method of Myers and Smith (1964), except that starch was dissolved in 1N KOH. The flow time was 133 sec for 1N KOH. Three separate measurements were performed for each starch type.

TABLE I Properties of Starches from 17 Mutant Genotypes of the Oh43 Inbred Line*

Genotype ^b	Amylose Content ^c (%)	Blue Value	λ _{max} (nm)	[η] (ml/g)	Swelling Power at at 85°C (%)	Solubility at 85° C (%)	% <i>T</i> at 650 nm
Normal	26.7	0.371	608	241	15.6	9.4	8.97
ae	46.0	0.677	610	125	9.7^{d}	23.1 ^d	0.62
bt1	24.9	0.369	615	218	12.0	7.0	3.41
bt2	24.7	0.323	613	237	e	· · · e	d
dul	30.5	0.434	611	157	11.8	13.6	9.23
h	28.1	0.388	616	234	14.9	10.0	8.55
sh2	30.1	0.351	610	238	10.6	3.6	2.37
sul	31.2	0.397	616	160	12.4	10.4	3.00
wx	0	0.063	542	268	62.1	25.7	56.83
ae bt1	32.4	0.306	598	139	· · · e	٠٠	1.72
ae dul	57.3	0.619	604	119	11.9^{d}	37.8d	0.85
dul sul	34.5	0.477	615	132	9.8	9.1	1.67
h sh2	26.4	0.394	614	228	15.3	9.8	8.86
h wx	0	0.109	560	257	40.7	15.1	31.09
sh2 bt1	27.7	0.367	617	228	9.6	3.3	1.95
sh2 wx	0	0.067	540	267	57.6	23.8	48.79
wx dul	0	0.061	540	266	56.2	25.4	49.29
LSDf	1.0	0.004	10	2	3.8	5.0	0.30

^a Values are the average of two determinations, except for $[\eta]$ which is the average of three determinations.

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and purification. The amount of starch isolated depended upon the amount of available corn of each genotype, but ranged from 5 to 100 g.

^b ae = amylose extender, bt = brittle, du = dull, h = horny, sh = shrunken, su = sugary, wx = waxy.

^c Percentage calculated from Fraction I of gel permeation chromatogram of isoamylase-debranched starch (Wang et al 1993).

d Measured at 100°C.

Sample not available for measurement.

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 $^{^{}f}$ P < 0.05.

Swelling Power and Solubility

Swelling power and solubility were determined at 85°C according to Leach et al (1959), but with these modifications. Starch (0.5 g for nonwaxy and 0.25 g for waxy, dry weight basis [dwb]) was added to 8 ml of distilled water in a 85°C water bath for 30 min and mixed with a stirring bar at moderate speed. The swelling power of amylose-extender (ae) and amylose-extender dull1 (ae du1) starches was measured at 100°C because of poor swelling at 85°C in a preliminary test. After 30 min of heating, the stirring bar was removed and rinsed with distilled water, and additional water was added to make the total water weight 10.0 g. The starch paste was centrifuged at $1,500 \times g$ for 20 min, after which 5 ml of supernatant was pipetted into a weighing dish and dried at 120°C for 2 hr to determine the soluble content. The remaining supernatant was carefully removed by suction and weighed to determine the amount of water absorbed by starch granules. Swelling power percentage was calculated with correction for solubles. The results were the average of two determina-

Both swelling power and solubility techniques have been used to reflect the arrangement of molecules within the starch granules. A starch with an extensive and strongly bonded structure, such as high-amylose starch, exhibits restricted swelling and dispersion. In contrast, waxy starch shows unrestricted swelling.

Percent Light Transmittance

The percent light transmittance (%T) of starch solutions in water (1%, w/w) was determined according to the method of Craig et al (1989). The solutions were heated in a boiling water bath and stirred for 30 min. After solutions were cooled to room temperature, the %T at 650 nm was measured against a water blank using an Hitachi U-2000 spectrophotometer (Hitachi Instruments, Inc., Tokyo, Japan). The results were the mean of two replicate samples.

Clarity, one of the important attributes of a starch paste, is a characteristic of starch source. Craig et al (1989) used %T as a measure of clarity. They proposed that when a beam of light passes through the native starch granules, most of the light is reflected back, and the starch seems white and opaque because the surface of the granule is larger than the wavelength of light.

Pasting Properties

Pasting characteristics of starch suspensions (6%, w/w, dwb), with the pH adjusted to 5.5, were measured using the Brabender Viscoamylograph (C. W. Brabender Instruments, Inc., South Hackensack, NJ) equipped with a 700-cm·g sensitivity cartridge operating at a bowl speed of 75 rpm. The temperature was raised from 30 to 95°C at a rate of 1.5°C/min, maintained at 95°C for 30 min, lowered to 50°C at the same rate, and held for 30 min. Because of a limited sample size, only one measurement was made on selected starch genotypes.

Gel Strength

The starch paste prepared with the Brabender Viscoamylograph was used to measure the gel strength after storing for one and seven days at 4°C. The paste from each starch genotype was poured into four aluminum dishes (27-mm i.d. × 27 mm). The rims of the pans were extended with aluminum foil to increase the height of the gel 1 cm above the rims (Takahashi et al 1989). The gel strength of the starch paste was measured at five different locations on each gel sample, and two gel samples per starch type were measured after one or seven days of storage using a Voland texture analyzer (Texture Technologies, Scarsdale, NY) as previously described (Wang et al 1992).

Thermal Properties

The thermal properties of starches were determined according to the method of Wang et al (1992), using a Perkin-Elmer DSC 7 analyzer equipped with a thermal analysis data station (Perkin-Elmer, Norwalk, CT). Three determinations were done on each starch genotype.

Statistical Analyses

Data of physicochemical properties among 17 maize genotypes were analyzed by using the SAS program (SAS 1990), and correlations were computed among properties and structural characteristics previously characterized (Wang et al 1993). Least significant differences were computed at P < 0.05.

RESULTS AND DISCUSSION

General Physicochemical Properties

The general properties of 17 mutant genotypes of the Oh43 inbred line and their least significant differences are summarized in Table I. Normal starch had an amylose content of 26.7%, a BV of 0.371, and a λ_{max} at 608 nm. The starches with higher amylose content, ae (46.0%), dul (30.5%), ae dul (57.3%), and dul sul (34.5%) (Wang et al 1993), had significantly greater BV than did normal starch, with the exception of ae bt1 starch, which had an amylose content of 32.4% and a BV of only 0.306. The λ_{max} range of nonwaxy starches was 598-617 nm. All waxy starches exhibited similar BV and λ_{max} values, except h wx starch, which had a significantly (P < 0.01) greater BV and λ_{max} than the other waxy starches. According to previous results (Wang et al 1993), starch from h wx showed little amylose content on an elution profile from gel permeation chromatography. This might account for the different BV and λ_{max} of h wx starch. The relatively small BV and λ_{max} of ae bt1 starch might be caused either by a great amount of intermediate material (22.5%) (Wang et al 1993) or by structural differences. Except for sh2 starch, the starches with amylose contents greater than 30% (ae, dul, sul, ae btl, ae dul, and dul sul) had $[\eta] < 200 \text{ ml/g}$, whereas waxy starches had $[\eta] > 250$ ml/g. Again, h wx starch showed different $[\eta]$ from other waxy starches. The standard deviation of the $[\eta]$ of all starches was <2. The shear rate was not determined, nor were the $[\eta]$ values verified as being in the Newtonian region.

Starches in this study were heated at 85°C to measure swelling power and solubility, with the exception of ae and ae dul starches, which were heated at 100°C because of their high amylose content and their consequent resistance to gelatinization (Wang et al 1993). Even when heated at 100°C, ae and ae dul starches exhibited less swelling power (9.7 and 11.9%, respectively) than normal starch (15.6%) heated at 85°C. The ae and ae dul starches showed significantly (P < 0.01) higher solubility than normal starch, presumably because the amylose, having a small molecular weight, leached out at 100°C. In contrast, the waxy starches showed unrestricted swelling and great solubility, probably because of the absence of a network structure from amylose molecules to hold the starch molecules together. The h wx starch had less swelling power and solubility than other waxy starches. The results corresponded to those for BV, λ_{max} , and $[\eta]$, suggesting the presence of associative bonding in h wx starch.

Results of the %T measurements differed greatly among samples. The ae starch had the smallest %T (0.62), and wx starch had the greatest (56.83). In general, nonwaxy starches had a %T < 10, whereas waxy starches had a %T > 30. Craig et al (1989) proposed that the disassociation of starch molecules during gelatinization diminished the reflecting ability of a starch granule and, thus, increased the %T of a starch paste. Swinkles (1985) suggested that naturally present lipid in the starch granules affects the clarity of starch pastes; the presence of amylose-lipid inclusion compounds makes starch pastes opaque or cloudy. In the present study, there were different %T values among waxy starches, which generally contain almost no lipid. Therefore, factors other than starch granular integrity and lipid content may also be important. Results from the present study confirm the hypothesis of Craig et al (1989), which states that the more starches swell, the greater the %T will be. Waxy starches, which exhibited unrestricted swelling, had greater %T than nonwaxy starches. Moreover, highamylose starches, which evidenced restricted swelling and dispersion, also showed relatively small %T.

Pasting Properties

The pasting properties of starches from some mutant genotypes

are listed in Table II. Because of the limited sample size, these tests were not performed on all starches. All waxy starches exhibited a high peak viscosity followed by a rapid decrease in viscosity that is attributable to the thinning effect from mechanical shearing. After cooling to 50°C (set-back viscosities), the waxy starch showed less viscosity than normal starch. The pasting temperatures also were lower than the temperature of normal starch. The high amylose content in ae starch restricted the swelling of starch granules, and, for this reason, no viscosity was observed under the test conditions. The dul starch had a low peak viscosity

TABLE II
Pasting Properties of Starches from Mutant Genotypes
of the Oh43 Inbred Line*

Genotype ^b		Peak Viscosity (BU)	Viscosity (BU) ^c				
	Pasting Temperature (°C)		at 95°C	at 95° C 30 min	at 50°C	at 50° C 30 min	
Normal	84.5	359	340	274	550	483	
ae ^d							
dul	90.3	101	70	80	101	100	
h	85.2	300	300	280	535	522	
wx	73.2	680	320	230	318	293	
h sh2	86.0	315	314	290	535	520	
h wx	72.0	620	340	229	352	310	
sh2 wx	72.3	673	330	228	326	294	
wx dul	74.6	622	311	212	312	290	

^a Starch concentration 6% (w/w, dry basis). Values from one determination.

^d No value observed.

TABLE III
Gel Strength of Some Starches from Mutant Genotypes
of the Oh43 Inbred Line^a

	Firmness	(g force)	Stickiness (g force)		
Genotype ^b	Day 1	Day 7	Day 1	Day 7	
Normal	5.4	6.1	0.5	1.0	
ae	0.8	1.8	0.2	0.5	
dul	3.4	10.4	1.2	2.1	
h	6.3	7.8	0.7	1.8	
wx	¢	0.8	c	0.4	
h sh2	6.4	8.0	0.8	1.7	
h wx	^c	0.8	с	0.5	
sh2 wx	с	0.9	c c	0.4	
wx dul	c	0.8	c	0.4	
LSD^d	2.0	2.0	2.0	2.0	

^a Values are the average of 10 determinations from two separate samples. ^b ae = amylose extender, du = dull, h = horny, sh = shrunken, wx = waxy.

that increased slightly during both heating and cooling. The pasting pattern of dul starch was attributed to its relatively high amylose content (30.5%) (Table I). This reinforced the bonding of the long chains of amylose within starch granules (Howling 1980) and resulted in a high pasting temperature (90.3°C) for dul starch. Normal, h, and h sh2 starches had similar pasting patterns.

Schoch and Maywald (1968) classified four types of starch according to pasting behavior. Type A starches swelled unrestrictedly during cooking and were unstable under shearing forces during heating. Waxy starches in the present study (wx. h wx, sh2 wx, and wx dul) were classified Type A, high-swelling starches. The normal, h, and h sh2 starches were classified Type B, starches that did not swell extensively and were not fragile under mechanical shearing. They showed high set-back viscosities, which indicates a degree of reassociation during cooling. Type C starch, a restricted-swelling starch, showed no pasting peak but, rather, exhibited a constantly high or an increasing viscosity during cooking. The dul starch was a Type C starch with quite a low viscosity that increased consistently during cooking and cooling. Type D starch had a high amylose content and evidenced very limited swelling; therefore, no viscosity was observed at normal concentrations. The ae starch in this test was classified Type D.

Gel Strength

The starch paste prepared in the viscoamylograph was used to evaluate the gel strength during refrigerated storage. Results are listed in Table III. In general, the gel strength increased during storage for seven days, but the extent of increase depended upon the starch source. Normal, h, and h sh2 starches showed similar values for both firmness and stickiness after one and seven days of storage. The waxy starches did not form gels after one day of storage and formed only very weak gels even after seven days of storage, probably because of a lack of amylose to form the network structure (Howling 1980, Ring et al 1987, Wang et al 1992). The very limited firmness and stickiness of the waxy starch gels after seven days indicated some reassociation. The ae starch, not fully gelatinized, had very low values for both firmness and stickiness. The dul starch gel was less firm than that of normal starch after one day of storage, but, after seven days, the dul starch gel became significantly firmer (P < 0.01) than those of all other starches, probably because of its high amylose content. Syneresis of water from the starch gel was observed for ae and dul starches after seven days of storage, reflecting their high amylose content.

Thermal Properties

The thermal properties of ae bt1, ae du1, and du1 su1 starches, measured using differential scanning calorimetry, are listed in Table IV, and the data for other starches were published elsewhere (Wang et al 1992). Perhaps because of their high amount of intermediate material, the ae bt1, ae du1, and du1 su1 starches had lower onset temperatures, broader gelatinization ranges, lower

TABLE IV
Thermal Properties of Starches from Mutant Genotypes of the Oh43 Inbred Line^a

	Gelatinization				Refrigerated-Storage Retrogradation			
Genotype ^b	7° (°C)	<i>R</i> ^d (°C)	$\Delta H_{\rm g}^{\rm e}$ (cal/g)	PHI	7 ₀ (°C)	<i>R</i> (°C)	$\Delta H_{\rm r}^{\rm g}$ (cal/g)	$r\%^{\rm h}$ $(\Delta H_{\rm r}/\Delta H_{\rm g})$
ae bt1	63.7	13.3	2.0	0.30	38.7	23.3	1.4	70.0
ae dul	65.6	16.0	1.2	0.15	41.2	25.6	0.8	66.7
dul su l	62.3	11.2	1.7	0.31	38.1	17.5	1.1	64.7

^a Values are the average of three determinations.

 $^{^{}b}$ ae = amylose extender, du = dull, h = horny, wx = waxy, sh = shrunken.

^c BU = Brabender units.

^c Gel too weak to support the probe.

^d P < 0.05.

 $^{^{}b}ae = \text{amylose extender}, bt = \text{brittle}, du = \text{dull}, su = \text{sugary}.$

^c Onset temperature.

^d Range of peak calculated as 2 ($T_p - T_o$), as described by Krueger et al (1987).

^e Enthalpy of gelatinization.

^e Peak height index = $\Delta H/(T_p - T_o)$, as described by Krueger et al (1987).

g Enthalpy of retrogradation.

h Ratio of enthalpy of retrogradation to enthalpy of gelatinization.

TABLE V

Correlations Between Structural Characteristics and Physicochemical Properties of Starches from 17 Mutant Genotypes of the Oh43 Inbred Line

Characteristic or Property	Amylose Content ^b	Intermediate Material Content	Chain Length at Fraction II of Debranched Amylopectin	Chain Length at Fraction III of Debranched Amylopectin	Fraction III/Fraction II of Debranched Amylopectin
Blue value	0.96**°	0.61**	0.56**	0.47**	-0.05
λ_{max}^{d}	0.81**	0.25	0.43*	0.05	0.25
$[\eta]^{\stackrel{\circ}{\mathfrak{c}}}$	-0.83**	-0.92**	-0.68**	-0.46**	0.02
Swelling power	-0.86**	-0.47	-0.54**	-0.13	-0.25
Solubility	-0.02	0.03	0.62**	-0.60**	0.40
$\%T^{\mathrm{f}}$	-0.88**	-0.49	-0.55*	-0.18	-0.14
Peak viscosity	-0.97**	-0.87**	-0.81**	-0.45	-0.18
Firmness of gel	0.61	0.40	0.56	-0.31	0.68*
Stickiness of gel	0.55	0.35	0.50	-0.33	-0.15
$T_{\rm o}^{\ g}$	-0.55*	-0.36	-0.31	0.27	-0.25
$\Delta H_{\rm g}^{\rm h}$	0.54*	0.51*	0.35	0.63**	-0.52*

^a Values and description are listed in Wang et al (1993).

TABLE VI Correlations Among Selected Physicochemical Properties of Starches from Mutant Genotypes of the Oh43 Inbred Line

Property	Swelling Power	Solubility	Peak Viscosity	Firmness of Gel	Stickiness of Gel
Blue value	-0.88**a	-0.07	-0.97**	0.55	0.50
λ_{max}^{b}	-0.98**	-0.55**	-0.87**	0.80**	0.76*
$[n]^{c}$	0.66**	-0.17	0.93**	-0.40	-0.37
$[\eta]^{^{\mathrm{c}}} \ \mathscr{U}^{\mathrm{d}}$	0.99**	0.46*	0.90**	-0.71*	-0.66
Swelling					
power		0.50**	0.92**	-0.76*	-0.71*

 $^{^{}a}$ * and ** = Significant at P < 0.05 and P < 0.01 levels of probability, respectively.

enthalpies, and lower peak height indices for gelatinization than did other starches reported previously (Wang et al 1992).

Correlation Analyses

Table V lists starches of 17 mutant genotypes and the correlations among their structural characteristics determined previously (Wang et al 1993) and their physicochemical properties measured in the present study. Amylose content was the most important attribute determining the physicochemical properties of starches. Amylose content was positively correlated with BV (r = 0.96) and λ_{max} (r = 0.81) and was negatively correlated with $[\eta]$ (r = -0.83), swelling power (r = -0.86), %T(r = -0.88), and peak viscosity on the viscoamylogram (r = -0.97), at a significance level of P < 0.01. The intermediate material content was negatively correlated with both $[\eta]$ (r = -0.92) and peak viscosity on the viscoamylogram (r = -0.87) (P < 0.01). The average chain length at fraction II of debranched amylopectin (long B chain of amylopectin) was negatively correlated with peak viscosity on the viscoamylogram (r = -0.81, P < 0.01). No other significant correlations with r values greater than 0.8 were found among other structural characteristics and physicochemical properties.

The high correlations between intermediate material content and $[\eta]$ and between intermediate material content and peak viscosity can be explained if the intermediate materials are indeed small molecules of amylopectin. Whistler and Doane (1961) reported that intermediate materials from starches of du su2, ae su1, and ae ae maize mutants had similar properties, such as

BV, iodine sorption capacity, and rate of retrogradation. More recently, the intermediate materials from amylomaize starch were characterized as having both low molecular weight branched molecules with four or five branches and an average chain length of 50 glucose units linked to a main linear chain of 100–150 glucoses (Baba and Arai 1984). Mercier (1973) suggested that differences in the chain length distribution within a population modified the solubility and other physical properties of a polysaccharide without necessarily altering the average chain length or the iodine-staining properties, so correlations among these properties are unexpected.

The correlations among selected physicochemical properties of starches from mutant genotypes of the Oh43 inbred line are summarized in Table VI. Swelling power was significantly (P < 0.01)correlated with both %T (r = 0.99) and peak viscosity on the viscoamylogram (r = 0.92) and was negatively correlated with BV (r = -0.88) and λ_{max} (r = -0.98). Crosbie (1991) reported that wheat starch swelling power was significantly (P < 0.01)correlated with starch paste peak viscosity (r = 0.81) and with total sensory score of boiled noodles made from the wheat starch (r = 0.88). He suggested that swelling power may be used as an alternative to starch paste viscosity for predicting noodle eating quality because of the relatively large sample size and long analysis time needed for determining paste peak viscosity with Brabender viscoamylography. The present data suggest that %T may be an appropriate alternative. Moreover, %T is easy to determine and needs only a small amount of sample. Peak viscosity also significantly correlated with BV (r = -0.97), λ_{max} (r = -0.87), and $[\eta]$ (r = 0.93). Firmness and stickiness of gels were significantly (P < 0.01) correlated (r = 0.98) to each other.

CONCLUSION

Amylose content was the most important structural characteristic affecting the physicochemical properties of starch from maize mutant genotypes. Amylose content significantly correlated with BV, λ_{max} , swelling power, %T, and peak viscosity. Although amylose content alone was the most important predictor of physicochemical properties, it did not explain the behavior of all starches. Among waxy starches, the h wx starch exhibited physicochemical properties different from those of other waxy starches. These differences suggest that the small amount of amylose present in h wx starch may be quite important in determining the properties of starch or the fine structure of amylopectin in h wx starch.

^b Values are from Fraction I of gel permeation chromatogram of isoamylase-debranched starch (Wang et al 1993).

^c* and ** = Significant at P < 0.05 and P < 0.01 levels of probability, respectively.

d Maximum absorbance wavelength.

^e Limiting viscosity number.

Percent light transmittance.

⁸ Onset temperature of gelatinization.

^h Enthalpy of gelatinization.

^b Maximum absorbance wavelength.

^c Limiting viscosity number.

d Percent light transmittance.

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