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Functional properties of starch from normal and mutant corn genotypes

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Abstract

The thermal and functional properties of starches from a wild-type corn, and *amylose extender25*, *dull39*, *sugary2* (*su2*), *and sugary1*(*su1*) corn mutants, all in the same (ExSeed68) genetic background were evaluated and related to their structural features obtained in a previous study. The onset temperature of gelatinization values of starches from all mutant lines ranged from 52.0 to 62.9 °C, temperatures that were all lower than that of the wild-type starch. The viscosity of the *su2* starch was relatively stable over the cooking process, showing only a small breakdown of the peak viscosity, suggesting high stability of starch granules against mechanical shear. The *su1* mutant starch formed the strongest gel among all starch-gel samples during measurements of both fresh and stored gel. Correlations were established between amylose percentage, chain-length distributions and pasting properties; Rapid Visco Analyser (RVA) and Differential Scanning Calorimeter (DSC) parameters; and DSC and texture analysis data.

Keywords: Corn starch; Structure-function; Starch pasting; Starch gels; Starch thermal properties; Starch gelatinization; Starch retrogradation; Starch texture; Corn mutants

1. Introduction

For new starches to be useful in food systems and other industrial applications, the functional properties, such as gelatinization, pasting, and retrogradation, should be fully understood. Starch functionality depends greatly on the molecular weight, size, and structure of the starch granule components, amylose (AM) and amylopectin (AP), which differ greatly in molecular-weight distribution and molecular structures. Variations in these molecular features influence pasting, retrogradation, viscoelastic, and rheological properties (Bahnassey & Breene, 1994; Morrison & Tester, 1991), which can have a major impact on the utilization of these starches in food products (Kobayashi, Schwartz, & Lineback, 1986; Yuan, Thompson, & Boyer, 1993). Starch structures differ within the same botanical source (Hizukuri, Takeda, Murata, & Juliano, 1989; Sanders, Thompson, & Boyer, 1990), but these differences are greater among starches from different botanical sources (Hizukuri, 1985, 1986; Lii & Lineback 1977; Whistler & Daniel, 1984). Differences among corn starches in granule swelling (onset of viscosity), peak temperature, peak viscosity, shear thinning during pasting, and gel firmness during storage, have been mostly attributed to differences in AP structure (Bahnassey & Breene, 1994; Doublier, Paton, & Llamas, 1987; Ring & Stainby, 1985), whereas differences in setback and final viscosity during pasting have been attributed to AM structure (Leloup, Colonna, & Buleon, 1991; Ott & Hester, 1965; Vasanthan & Hoover, 1992).

Some endosperm mutants of corn (*Zea mays* L.), such as *amylose-extender* (*ae*), *dull* (*du*), *sugary-1* (*su1*), and *sugary-2* (*su2*), impact the AM:AP ratio of the starch, the specific structures of AM and AP, and, potentially, the functional properties of the starch. For example, starch from amylomaize, a corn endosperm mutant containing the *ae* mutant gene, has an apparent AM content of up to 80% (Banks & Greenwood, 1975). In addition to the high apparent AM content, *ae* starches contain branched molecules with a higher proportion of longer chains (DP > 30) than is found in the AP of common corn starch

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(Klucinec & Thompson, 1998; Takeda, Takeda, & Hizukuri, 1993).

The relative AM content of starch in *du1* mutant kernels ranges from slightly to greatly higher than normal, depending on the genetic background (Shannon & Garwood, 1984). Starch granules from *du1* mutants seem to have normal structural and physical properties, although some abnormally shaped granules are found in the mutant endosperm (Shannon & Garwood, 1984).

The *su2* starch granules have a slightly greater percentage of apparent AM (29 vs 21%) and a lower gelatinization temperature than does normal, commercially available, corn starch (Kramer, Pfahler, & Whistler, 1958; Pfahler, Kramer, & Whistler, 1957; Li & Corke, 1999; Perera, Lu, Sell, & Jane, 2001; White, Pollak, & Johnson, 1994), and suitable pasting properties for application in starch-thickened acidic foods (White et al., 1994). The *su2* starches also retrograde less during storage than do normal starches (Campbell, White, & Pollak, 1994; Inouchi, Glover, Sugimoto, & Fuwa, 1991; White et al., 1994), and have less swelling power than does normal corn starch (Li & Corke, 1999). Also, *su2* starch has an improved nutritional quality as a result of its high susceptibility to α-amylase digestion; thus, its use has been suggested in improving animal feed value (Sandstedt, Strahan, Ueda, & Abbot, 1962).

The *su1* mutants of corn accumulate, in addition to starch, a novel form of water-soluble polysaccharide, termed phytoglycogen (Summer & Somers, 1944). The *su1* maize kernels are wrinkled, have reduced amounts of dry material, with the concentration of sugars being greater and the starch content much less than in normal corn (Creech, 1965). Yeh, Garwood, and Shannon (1981) reported widely different apparent AM percentages (0 and 65%) in the starches from *su1* mutants placed in corn of different backgrounds. The great differences, however, were likely caused not only by genetic background, but also by different environmental conditions during kernel development, kernel age, and methods of starch isolation and AM measurement during testing.

Although some structural features of the starch components for many corn endosperm mutants in a few genetic backgrounds have been characterized, and some functional features examined, variations in both structure and the related functional properties of these mutants, all in the same genetic background, have not been fully examined. Furthermore, the experimental corn line, ExSeed68, with mutants introduced, has had very little evaluation of these starch properties. Thus, the full impact of genetics on the corn-mutant starches is not known. Therefore, the objectives of this study were to characterize the thermal and functional properties of the starch from a wild-type (normal) corn starch and *amylose extender25*, *dull39*, *sugary2*, *and sugary1* corn mutants in the ExSeed68 genetic background and to relate these properties to the previously determined structural features of the starches.

2. Materials and methods

2.1. Starch

Corn (*Zea mays* L.) kernels from the ExSeed68 line [wild type (normal) and *dull*39 (*du39*), *amylose extender25* (*ae25*), *sugary2* (*su2*), *sugary1* (*su1*) genotypes] were provided by ExSeed Genetics, LLC (Ames, IA, USA). All corn endosperm mutants were developed from the normal corn and grown in summer 1999 under the same environmental conditions near Ames, IA, USA.

2.2. Starch isolation

Starch was extracted from the corn kernels by using the modified 100-g procedure previously described by Singh, Johnson, Pollak, Fox, and Bailey (1997). The extraction procedure was performed twice for all starch types, except for the *su1* mutant, which was available only in limited quantity; thus only one extraction of 50 g was performed.

2.3. X-ray diffraction analysis

X-ray diffraction patterns of the starches were obtained by using a Scintag XDS-2000 diffractometer with a 2.2 kW line-focus copper tube (Thermo ARL, Switzerland) and a KEVEX 4461 detector (KEVEX X-ray, CA, USA) according to Perera et al. (2001).

2.4. Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC) was used to analyze thermal properties of the starches. Pyris software for windows package (V2.04, Perkin–Elmer, Norwalk, CT, USA) was employed by using procedures previously described (Seetharaman, Tziotis, Borras, White, Ferrer, & Robutti, 2001; White, Abbas, & Johnson, 1989). The samples were subjected to a temperature scan from 30 to 110 °C in aluminum pans (30–180 °C in stainless-steel pans for the *ae25* mutant starch to fully gelatinize this starch) at 10 °C min^{-1}. The *ae25* starch was cooled immediately to 30 °C and then rescanned under the same conditions as the first heating, allowing the determination of the AM–lipid complex, which overlapped (for the *ae25* starch) with the gelatinization of the AP peak during the first heating, but was formed alone during the second scan. Thus, Peak I, and all its properties, was defined as the difference between the peaks found during the first temperature scanning and the peak (Peak II) found during the immediate rescanning of the starch. To measure retrogradation properties, the gelatinized starch in the DSC pan was stored for 7 days at 4 °C, then equilibrated at 25 °C for 1.5 h before being reanalyzed in the DSC at a temperature range of 30–110 °C (30–180 °C for the *ae25* mutant starch) at 10 °C min^{-1}. Starch samples from duplicate extractions were analyzed in

duplicate, and the averaged results reported. Starch thermal properties, such as peak onset temperature (T_o), peak range (R), and change of enthalpy (δH) for all peaks [starch gelatinization (Peak I during gelatinization), melting of retrograded starch (Peak I during retrogradation), AM–lipid complex (Peak II during gelatinization and retrogradation), and AM peak (for *ae25* starch, Peak III during gelatinization and retrogradation)], were computed automatically.

2.5. Pasting properties

The pasting properties of the starches were analyzed with a Rapid Visco Analyser (RVA) model 4 (Newport Scientific Pty. Ltd, Warriewood, NSW, Australia) and STD2 temperature profile equipped with Thermocline for Windows software (V1.2) (Takahashi & Seib, 1988). Two RVA profiles were obtained for each sample and the results were averaged. Pasting temperature (P_{Temp}), peak viscosity (PV), hot paste viscosity (HPV), final viscosity (FV), breakdown (difference between PV and HPV), and setback (difference between HPV and FV) were recorded.

2.6. Gel strength

The starch pastes from the RVA analysis were analysed for gel strength with a Texture Analyzer (Stable Micro Systems TA.XT2, Texture Technologies Corp., Scarsdale, NY, USA) equipped with Texture Expert for Windows software (V1.22) (Takahashi & Seib, 1988). The starch gels obtained from the RVA analyses were stored for 1 day at 25 °C and 7 days at 4 °C. Gel strength and adhesiveness data are averages of five readings per gel, for each storage condition. The peak force is reported as the gel strength at each storage condition.

2.7. Statistical analysis

A complete random design was employed for the study of the starch samples. Functional property data from the current paper were correlated to structural data (characterization of AM and AP ratios and branch-chain lengths) on these same starches, obtained by fractionation of starches by gel-permeation chromatography reported in a previous study (Tziotis et al., 2004). Analysis of variance (ANOVA), correlation analyses (SAS Institute, Cary, NC, USA), and the *t*-test were used to compare means of data and to determine least significant difference (LSD) at $\alpha = 0.05$. Significant values were accepted at $P < 0.05$ unless otherwise indicated. The minimum r value for correlations to be significant at the $P < 0.05$ level was $r = 0.93$ (Steele & Torrie, 1980).

3. Results and discussion

3.1. X-ray diffraction analysis

The X-ray diffraction patterns (Figs. 1–5) of the samples revealed an A-type structure for the wild, *du39*, *su2* and *su1* mutant starches. A B-type structure was observed for *ae25* mutant starch that is typical of high-amylose starches as previously reported (Zobel, 1988). The normal (wild) corn starch had an A-type diffraction pattern, consistent with previous reports for normal corn starch (Perera et al., 2001; Zobel, 1988), the *su2* starch had a weak A-type diffraction pattern, indicating weak crystallinity, in agreement with previous findings (Campbell et al., 1994; Perera et al., 2001), and the *su1* starch had an A-type diffraction pattern similar to that of sweet rice (Jane et al., 1999). The *du39* starch had a weaker than normal A-type diffraction pattern as previously reported for the *du* mutant in an Oh43 background (Inouchi, Glover, Sugimoto, & Fuwa, 1984). The greater amylose contents of *su2* and *du39* starches are possibly responsible for their weaker X-ray diffraction patterns (Inouchi et al., 1984).

3.2. Structural data on branch-chain lengths of starches

In a previous study, we characterized the ratios of amylose and amylopectin in all of the starches presented in the current paper. The average apparent AM contents of the starch from the wild type, *du39*, *ae25*, *su2*, and *su1* corn were 21.0, 28.6, 51.9, 29.7, and 25.4%, respectively (Tziotis et al., 2004). We also compared branch-chain lengths of all the starches by three separate methods described at length in the previous study (Tziotis et al., 2004). A brief summary is included (Table 1) of the results from the fractionation of all starches by one procedure, gel-permeation chromatography (GPC), based on molecular size distribution of the starch. The data from the GPC procedure were similar to data obtained from the other two methods. Briefly, in the GPC method, fractions from all starch types were collected according to the total carbohydrate (CHO) profile and divided into those of large (L), medium (M), and small (S) molecular weight. These fractions were enzymatically

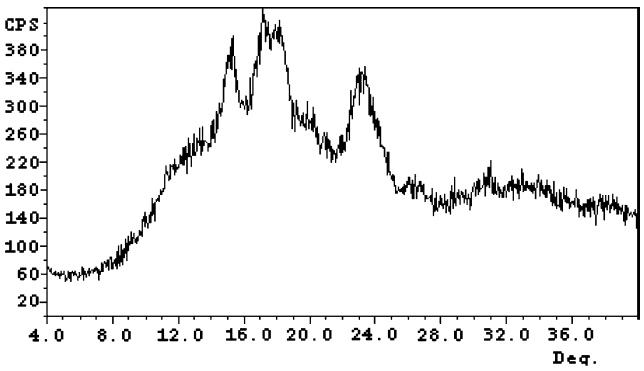

Fig. 1. X-ray diffraction pattern of wild type corn starch.

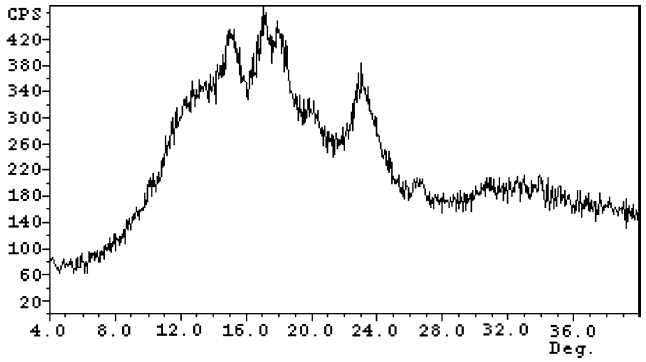

Fig. 2. X-ray diffraction pattern of *du39* mutant corn starch.

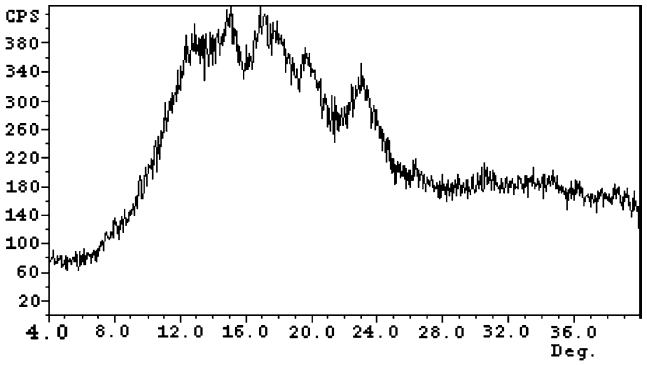

Fig. 4. X-ray diffraction pattern of *su2* mutant corn starch.

debranched, and the percentage chain-length distributions, weight-chain-length average, and greatest degree of polymerization (DP) were reported. The values in Table 1 were used to calculate correlation values related to the functional data obtained in the current paper.

3.3. DSC of native starch

3.3.1. Gelatinization (Peak I)

The T_o values of starches from all mutant lines ranged from 52.0 °C for *su2* to 62.9 °C for *du39* (Table 2), temperatures that were all lower than that of the normal starch (64.5 °C), which agrees with relative values reported by others for these mutants in the same (Perera et al., 2001) and other genetic backgrounds (Brown, Creech, & Johnson, 1971; Campbell et al., 1994; Kramer et al., 1958; Li & Corke, 1999; Ng, Duvick, & White, 1997; Ninomya, Okuno, Glover, & Fuwa, 1989; Pfahler et al., 1957). Both absolute and relative thermal property values of corn starches can vary, however, based on their genetic backgrounds, as indicated by the following reports on T_o values. The T_o values also may vary among studies according to the heating procedures (DSC parameters). For example, the *du* starch in the IA5125 inbred line had a T_o of 61.0 °C, the *ae* starch had a T_o of 68.8 °C, and normal starch a T_o of 64.2 °C (Sanders et al., 1990). Similarly the T_o for the *ae* starch in the W64A background (70.6 °C) was greater than that of normal starch (63.9 °C) (Krueger, Walker, Knutson, & Inglett, 1987). The *su2* mutant in an Oh43 background had a variable T_o, which decreased with increased dosage of *su2*, reaching 58.3 °C (normal 67.3 °C) for the complete *su2* mutant background (Campbell et al., 1994). Alternatively, Inouchi et al. (1991) reported that normal corn starch in the Oh43 background had a T_o of 61 °C, *ae* (65 °C), *du* (64 °C), and *su2* (45 °C); thus, all mutant starch values in that study except for *su2* were greater than that of the normal starch. Wang, White and Pollak (1992) also reported DSC values for corn mutants in the Oh43 inbred line with slightly greater relative and absolute T_o values than those of Inouchi et al. (1991). The T_o of the *ae*, *du*, and *su1* starches were 68.7, 67.2, and 64.6 °C, respectively, with the normal counterpart T_o being 67.2 °C.

Li and Corke (1999) studied corn starch from five different genetic backgrounds (A632, Oh43, Hz85, Hz101, and Hz47) consisting of the *du* and *su2* starches and their normal counterparts. The properties of *du* starch were very similar to those of the normal starch, as noted by Wang et al. (1992), and the *su2* starch had T_o values approximately 10 °C lower than those of the normal. The *su1* mutant corn starch in the P39-5XP51-B background had a T_o of 62 °C, with the normal counterpart at 65 °C (Ninomya et al., 1989). A low T_o value is potentially desirable for food processing because of energy savings and nutrient and flavor retention associated with the lower temperatures required for starch thickening. A correlation analysis between the DSC parameters and the GPC branch-chain-length distributions

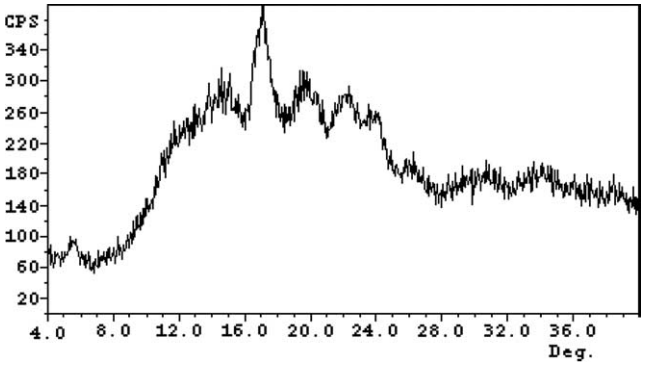

Fig. 3. X-ray diffraction pattern of *ae25* mutant corn starch.

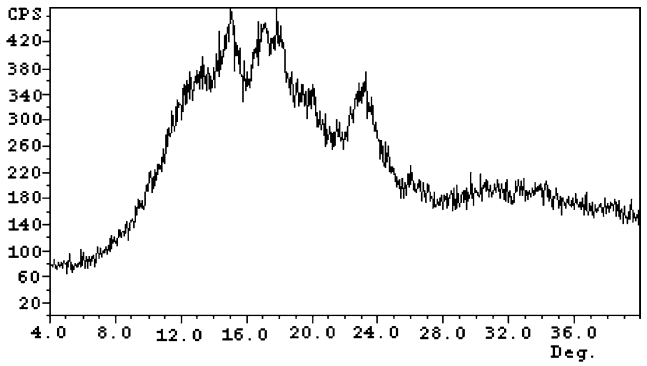

Fig. 5. X-ray diffraction pattern of *su1* mutant corn starch.

Table 1
Summary of structural data from Tziotis et al. (2004) branch-chain-length distributions of large, medium, and small MW fractions of starches obtained with gel-permeation chromatography

Sample	$DP_{Peak\ I}$ [a]	$DP_{Peak\ II}$	% Distribution					Greatest DP
			DP 6–12	DP 13–24	DP 25–36	DP>36	$\overline{CL}_w$ [b]	
Wild L	13	46	18.0±0.9	45.7±1.0	13.2±0.5	23.1±1.4	36.2	75
Wild M	13	46	20.9±0.6	45.8±0.9	14.5±0.2	18.8±1.7	38.8	66
Wild S	13	46	26.6±0.6	53.3±0.2	11.3±0.3	8.7±0.6	36.2	58
du39 L	13	47	19.2±0.1	50.7±0.4	14.6±0.1	15.5±0.6	34.1	72
du39 M	13	45	20.3±0.1	48.5±1.5	15.3±0.2	15.7±1.2	37.3	66
du39 S	12	44	26.0±0.1	55.9±1.1	12.2±0.5	5.9±0.8	34.9	58
ae25 L	15	49	9.2±0.4	36.6±0.1	14.6±0.3	39.6±0.0	44.7	80
ae25 M	15	50	9.3±0.2	37.2±0.0	16.2±0.1	37.4±0.4	43.0	83
ae25 S	16	46	16.7±0.8	46.9±3.4	17.6±0.9	18.7±3.5	36.8	72
su2 L	12	37	24.9±0.0	41.5±0.1	15.1±0.0	18.5±0.2	36.3	69
su2 M	12	43	27.9±1.6	41.4±0.9	14.0±0.5	16.7±0.0	33.8	71
su2 S	11	47	34.8±0.4	43.4±0.1	12.7±1.2	9.2±0.7	38.0	54
su1 L	12	39	21.4±0.3	45.9±0.0	14.5±0.4	18.2±0.1	36.0	70
su1 M	12	39	21.5±0.4	45.5±0.9	14.5±0.2	18.5±0.4	39.5	64
su1 S	11	40	29.8±0.2	39.4±0.3	13.9±0.5	16.9±0.9	36.0	56

Numbers reported are averages of two replications±standard deviation. L=large molecular weight, M=medium molecular weight, S=small molecular weight.
[a] DP_{Peak}=degree of polymerization at the maximum height of the peak.
[b] CL_w=weighed-average chain-length.

of the starches (Table 3) revealed that the percentage of branch chains with DP 6–12 was negatively related to T_o ($r=-0.97$, $P<0.05$) indicating that an increased percentage of short chains contributed to weaker starch crystallinity, resulting in starches with lower T_o.

The *R* values of the starches from the mutant lines were greater than that of starch from the wild type, a finding also noted in previous work for *du39* (Li & Corke, 1999), *ae25* (Klucinec & Thompson, 1999; Krueger et al., 1987; Ng et al., 1997; Sanders et al., 1990), *su2* (Campbell et al., 1994; Li & Corke, 1999), and *su1* (Ninomya et al., 1989) mutants. Wide *R* values could be optimal for food processes requiring several sequential 'events' during starch cooking, because the food system could maintain a consistent and stable viscosity over a wide temperature range. The *ae25* starch had the broadest endotherm ($R=16.8$ °C), a feature previously noted for *ae* starches by many other researchers (Inouchi et al., 1984; Kasemsuwan, Jane, Schnable, Stinard, & Robertson, 1995; Klucinec & Thompson, 1999; Krueger et al., 1987; Ng et al., 1997; Sanders et al., 1990; Wang et al., 1992). The *R* of this starch was wider than those of the other starch types because of the greater amount of amorphous regions in the starch granule caused by the AM. Furthermore, the smaller amount of AP crystallites in the granules and the distance between the molecules did not allow cooperative melting (Sanders et al., 1990).

The δH value of starch from *su2* (7.7 J g^{-1}) was less than that of the wild type starch (14.1 J g^{-1}), values that were relatively and absolutely comparable to data reported in the literature (Campbell et al., 1994; Inouchi et al., 1991; Li & Corke, 1999; Perera et al., 2001). The *du39* starch (16.1 J g^{-1}) had a greater δH value, and *ae25* starch (15.7 J g^{-1}) tended to be greater than did starch from wild type corn, a trend also shown by Sanders et al. (1990) and Wang et al. (1992). Our lower δH value of *su1* mutant starch (8.5 J g^{-1}) agreed with that reported by Wang et al. (1992). The low δH value of *su2* starch may be attributed to the low crystallinity content noted by the X-ray diffraction analysis,

Table 2
Differential scanning calorimetry thermal properties of starch from wild type corn and mutants, *du39*, *ae25*, *su2*, and *su1*

Sample	Peak I			Peak II		
	T_o (°C)	*R* (°C)	δH (J g^{-1})[a]	T_o (°C)	*R* (°C)	δH (J g^{-1})[a]
Wild type	64.5a	8.4e	14.1b	85.1b	18.5c	2.0b
*du*39	62.9b	9.8d	16.1a	85.2b	17.6cd	2.5b
*ae*25	62.5b	16.8a	15.7ab[b]	79.3c	27.7a	2.6b
su2	52.0d	13.1b	7.7c	79.4c	22.4b	3.6a
su1	60.4c	11.3c	8.5c	87.2a	16.1d	2.0b

Values reported are means of four replicates (two from each extraction, except for *su1* mutant starch, for which four replicates of one extraction were conducted). Numbers followed by the same lower case letter within each column are not significantly different at $P<0.05$.
[a] Peak I: endotherm attributed to loss of native starch double helices. Peak II: endotherm attributed to melting of AM–lipid complexes.
[b] Value reported for *ae25* starch is the sum of δH obtained from both Peak I and a third peak not reported in the table.

Table 3
Correlation coefficients among structural and functional parameters of starches

	T_oG-I	P_{Temp}	PV	HPV	FV	Breakdown	Setback	Gel strength 1	Gel strength 7
%AM[a]		0.99*		−0.99*	−0.99**				
DP 6–12	−0.97*								
DP 25–36		0.98*	−0.99**	−0.99*	−0.98*		−0.97*		
T_oG-II								0.98*	
RG-II								−0.96*	0.98*
δHG-II								−0.96*	
T_oR-II						0.96*			
δHR-II		0.96*							
%R				0.96*	0.96*		0.96*		
P_{Temp}				−0.99**	−0.99**		−0.99**		
PV		−0.98*		0.98*	0.97*		0.96*		
HPV					0.99***		0.99**		
FV							0.99***		

Horizontally: from Differential Scanning Calorimeter, ToG-I = onset temperature of Peak I during gelatinization temperature scanning; From Rapid Visco Analyser; P_{Temp}, pasting temperature. PV, peak viscosity; HPV, hot paste viscosity; FV, final viscosity; Breakdown = PV − HPV; Setback = FV − peak viscosity; From Texture Analyzer, gel strength on day 1, and gel strength on day 7 of storage. *, **, *** = $P < 0.05$, 0.01, 0.001, respectively.

[b] Vertically: AM = % amylose; DP = degree of polymerization of branch-chains; From Differential Scanning Calorimeter, ToG-II = onset temperature of Peak II during gelatinization temperature scanning, RG-II = range of Peak II during the gelatinization temperature scanning, δHG-II = enthalpy change of the AM–lipid complex (Peak II) during the gelatinization temperature scanning, ToR-II = onset temperature of Peak II during the retrogradation temperature scanning, δHR-II = enthalpy change of AM–lipid complex (Peak II) during the retrogradation temperature scanning, *%R* = percentage of retrogradation; from Rapid Visco Analyser, Horizontally: from Differential Scanning Calorimeter, ToG-I = onset temperature of Peak I during gelatinization temperature scanning; from Rapid Visco Analyser; P_{Temp}, pasting temperature. PV, peak viscosity; HPV, hot paste viscosity; FV, final viscosity; Breakdown = PV − HPV; Setback = FV − peak viscosity; From Texture Analyser, gel strength on day 1, and gel strength on day 7 of storage.

and loose packing of the branch chains noted by the lower T_o (52.0 °C), observations also suggested by Campbell, White, and Pollak (1995), Fuwa et al. (1987) and Inouchi et al. (1984). The low δ*H* of *su2* and *su1* starches indicated that they require less energy for starch gelatinization to occur, again resulting in a potential energy savings.

3.3.2. AM–lipid complex (Peak II)

The *su1* mutant had the greatest T_o value for this peak among all starches (Table 2) suggesting a tighter association of the AM–lipid complex. The T_o values for the wild type and *du39* mutant starches were not significantly different from each other, and those of the *ae25* and *su2* starches both at a similar value, were lowest. The *ae25* mutant had the widest *R* among all starches. The δ*H* value of Peak II for *su2* starch was greater than those of other starches indicating the greater amount of lipids present (Gudmundsson, 1994) in this starch type as also reported by Perera et al. (2001).

3.3.3. Amylose peak (Peak III)

The only starch for which a third peak was observed after temperature scanning from 30 to 180 °C was the *ae25* starch. The increased AM percentage of this starch was responsible for this distinct peak, because these molecules form tighter associations requiring higher temperatures to complete gelatinization (Boltz & Thompson, 1999; Sievert & Holm, 1993). The average T_o for Peak III of the *ae25* starch was 118.8 °C, its *R* was 12.4 °C, and the δ*H* was 0.3 J g^{-1} (data not shown). For the *ae25* starch, the δ*H* value of Peak I reported in Table 2 is the sum of the δ*H* values for Peak I and III as both Peak I and III contribute to the gelatinization of starch, especially during processes involving temperatures greater than 100 °C (Kasemsuwan et al., 1995).

3.4. DSC of retrograded starch

3.4.1. Melting of retrograded starch (Peak I)

The T_o values for the melting of the retrograded mutant starches, except for *su2*, were greater than that of the wild type starch (Table 4). The *R* values were different among all starches with values ranging from 13.0 °C for *su1* starch to 39.7 °C for *ae25* starch. The δ*H* values of starches from the mutant lines, except for *ae25*, were lower than that of the wild type starch.

The low δ*H* value for *su2* and *su1* retrograded starches were partly a reflection of their low δ*H* value for gelatinization; however, the percentage of retrogradation (*%R*) values also were low, suggesting that *su2* and *su1* starches were subject to a minimal amount of aligning and recrystallization (Campbell et al., 1994; White et al., 1989), which results in a starch stable to changes during frozen and refrigerated processes. Thus, these starches would be desirable for stored and frozen food products. The *du39* starch also had a relatively low δ*H* for retrogradation and *%R*.

3.4.2. AM–lipid complex (Peak II)

The T_o values (retrogradation) for Peak II of all mutant starches were lower than that of the wild type starch (Table 4). The T_o values for the wild type, *du39*, and *su2* starches tended to be greater than the T_o for the same peak

Table 4
Differential scanning calorimetry thermal properties of retrograded starch from wild type corn and mutants, *du39*, *ae25*, *su2*, and *su1*

Sample	Peak I				Peak II		
	T_o (°C)	R (°C)	δH (J g^{-1})[a]	%R	T_o (°C)	R (°C)	δH (J g^{-1})[a]
Wild type	38.5d	19.3d	8.2a	58.5a	88.9a	12.2d	1.6d
*du*39	40.6c	28.7b	5.2b	33.3c	87.1b	14.9c	2.8b
*ae*25	43.2b	39.7a	8.1a[b]	57.5a	70.1e	28.2a	1.8d
su2	39.6cd	20.2c	2.5c	33.1c	84.4d	16.8b	3.6a
su1	46.4a	13.0e	3.2c	38.1b	86.1c	16.3b	2.2c

Values reported are means of four replicates (two from each extraction, except for *su1* mutant starch, for which four replicates of one extraction were conducted). %R = % retrogradation = (enthalpy change of melting of retrograded starch/enthalpy change of gelatinization of native starch) × 100. Numbers followed by the same lower case letter within each column are not significantly different at $P < 0.05$.

[a] Peak I: endotherm attributed to melting of retrograded starch double helices. Peak II: endotherm attributed to melting of AM–lipid complexes.

[b] Value reported for *ae25* starch is the sum of δH obtained from both Peak I and a third peak not reported in this table.

during the first scanning of the starches, suggesting that a reorganization of the AM–lipid complex took place during 7-day storage. The *ae25* starch had the greatest R value, whereas *su2* starch had the greatest δH among all types.

3.4.3. Amylose peak (Peak III)

As noted earlier, the only starch for which a third peak was observed was the *ae*25 (Sievert & Pomeranz, 1989). Its average T_o for the melting of the retrograded starch was 154.0 °C, its R was 19.1 °C, and the δH was 1.1 J g^{-1}. As for gelatinization, the value reported for Peak I in Table 4 is the sum of the δH values for Peak I and III, as both Peak I and III contribute to properties of the retrograded starch, especially during processes involving temperatures greater than 100 °C. The T_o and δH values during retrogradation were greater than the values for gelatinization. Perhaps the first heating to 180 °C served as an annealing process for the amylose crystals found in this starch (Sievert & Pomeranz, 1989; Sievert & Wursch, 1993). As previously suggested (Boltz & Thompson, 1999), the annealed amylose, upon reheating, melts at a higher temperature. Retention of some nucleation sites of the amylose crystals could explain the shifting of the third endotherm peak to a higher temperature, and the greater δH observed during retrogradation than during gelatinization of the *ae25* starch.

3.5. Pasting properties of native starches

The *ae25* starch did not become thick during pasting with the RVA, as expected, because the temperature range (50–95 °C) of the process did not allow the gelatinization of AM, the principal component of *ae25* starch; thus, no data is reported. Assignment of statistical differences was not possible with two replicate analyses; however, the standard deviations for all duplicate values were very close, indicating good agreement among duplicates. The P_{Temp} of each of the mutant starches, ranging from 86 to 93 °C (Table 5), were greater than that of the wild type starch (78 °C). The *du39* starch displayed a high P_{Temp} (89 °C), possibly resulting from the increased bonding of AM molecules within the granule as suggested by Wang et al. (1992), which could also explain the increased T_o of DSC Peak II for the native *du39* starch. Values for all pasting profiles, except P_{Temp}, generally were greater for the wild type starch than for the *du39*, *su2*, and *su1* native starches. The high PV displayed by the wild type starch may be attributed to its high AP concentration (79%) (Zeng, Morris, Batey, & Wrigley, 1997), a value greater than that of the mutant starches as shown by Tziotis et al. (2004). There was a marked thinning of the starch viscosity during the cooking period for all starch types, as indicated from the breakdown values.

The *su2* starch pasting profile showed a high P_{Temp}, a low PV, and low breakdown, results in agreement with those reported previously regarding *su2* mutant starches in various genetic backgrounds (Campbell et al., 1995; Li & Corke, 1999; Perera et al., 2001). Also, it has been suggested (Brown et al., 1971; Campbell et al., 1994) that *su2* starch may have a loosely branched AP structure responsible for these properties. The viscosity of the *su2* starch over the process was relatively stable, showing only a small breakdown of the PV during cooking, and suggesting a high stability of starch granules against mechanical shear. Perhaps the greater apparent AM content (29.7%; Tziotis et al., 2004) and the greater amount of lipids reportedly

Table 5
Pasting properties of starch from wild type corn and mutants, *du39*, *ae25*, *su2*, and *su1* as measured on a Rapid Visco Analyser

Sample	P_{Temp} (°C)	PV (RVU)	HPV (RVU)	FV (RVU)	Breakdown (RVU)	Setback (RVU)
Wild type	78 ± 0.0	153 ± 0.3	95 ± 0.4	195 ± 0.9	58 ± 0.4	101 ± 1.0
*du*39	89 ± 0.3	79 ± 0.2	33 ± 0.5	61 ± 0.7	46 ± 0.2	28 ± 0.1
su2	93 ± 0.5	41 ± 0.4	15 ± 0.1	29 ± 0.5	26 ± 0.3	14 ± 0.6
su1	86	80	49	101	31	52

Values are means of two replicates ± standard deviation except for *su1* mutant with only one replication. P_{Temp}, pasting temperature. PV, peak viscosity; HPV, hot paste viscosity; FV, final viscosity; Breakdown = PV − HPV, Setback = FV − PV.

present (Morrison, Tester, Snape, Law, & Gidley, 1993; Perera et al., 2001; Tester & Morrison, 1990) in this starch type may result in less breakdown, because of restricted granule swelling. The apparent AM content (25.4%; Tziotis et al., 2004) of the *su1* starch compared to wild type and other mutant starches likely explains its behavior, which was generally intermediate for all RVA parameters measured.

The higher setback value of the wild type starch is associated with a greater tendency of the starch to retrograde (Wang & White, 1994), also evident from the DSC %*R* data and the correlation established between these parameters ($r=0.96$, $P<0.05$, Table 3). There was a positive correlation between AM percentage and P_{Temp} ($r=0.99$, $P<0.05$), as also noted by Wang et al. (1992). The AM–lipid interaction likely restricted swelling of starch, thus delaying the pasting temperature. The AM percentage was negatively correlated with HPV ($r=-0.99$, $P<0.05$) and FV ($r=-0.99$, $P<0.05$), because its presence inhibits the development of viscosity during cooking. The chains with DP 25–36 correlated positively with the P_{Temp} ($r=0.98$, $P<0.05$), and negatively with PV ($r=-0.99$, $P<0.01$), HPV ($r=-0.99$, $P<0.05$), FV ($r=-0.98$, $P<0.05$) and SB ($r=-0.97$, $P<0.05$), indicating a negative role in delaying and limiting the viscosity development of the starches.

Among the RVA parameters, there were negative correlations between P_{Temp} and PV ($r=-0.98$, $P<0.05$), P_{Temp} and HPV ($r=-0.99$, $P<0.01$), P_{Temp} and FV ($r=-0.99$, $P<0.01$), and P_{Temp} and setback ($r=-0.99$, $P<0.01$, Table 3). These correlations may be explained by the AM–lipid complex, which not only delayed the P_{Temp} because of restricted swelling, but reduced the values of the other parameters. Also, there were positive correlations between PV and HPV ($r=0.98$, $P<0.05$), PV and FV ($r=0.97$, $P<0.05$), PV and setback ($r=0.96$, $P<0.05$), HPV and FV ($r=0.99$, $P<0.001$), HPV and setback ($r=0.99$, $P<0.01$), and FV and setback ($r=0.99$, $P<0.001$), because the structural characteristics of the starch molecules that dictated the development of viscosity also influenced these RVA parameters.

The P_{Temp} from RVA and δH of Peak II during DSC retrogradation, positively correlated ($r=0.96$, $P<0.05$, Table 3), may be explained by the AM–lipid complex (and higher %AM), which delays the pasting of the starch gel, and also allows formation of a more stable structure after 7-day storage. The positive correlation of breakdown (by RVA) and T_o of Peak II (by DSC) during retrogradation ($r=0.96$, $P<0.05$) might be because a high T_o suggests a highly organized complex with more tendency to retrograde.

3.6. Textural properties of native starches

In contrast to results reported by Wang et al. (1992) where the *su1* mutant starch in the Oh43 genetic background developed a gel too weak to measure, the *su1* mutant starch in the current study formed the strongest gel among all starch-gel samples (Table 6). The *ae25* mutant starch did not form a gel, because it was unable to fully cook within the temperature range used during the RVA analysis; thus, values are not included. Greatly lower gel-strength values were obtained for *su2* starch than for all other starches after 1-day storage, in agreement with data reported by Campbell et al., 1994). The gel strength usually decreases with increased starch-lipid content (Takahashi & Seib, 1988), because the lipids likely hold the AM within the starch granule, thus decreasing the AM concentration in the continuous phase, and reducing gel firmness as previously observed for the *su2* mutant starch (Campbell et al., 1994; Wang et al., 1994).

Table 6
Gel-strength and adhesiveness of starch from wild type corn and mutants, *du39*, *ae25*, *su2*, and *su1* measured on a TA-XT2 Texture Analyser

Treatment	Wild type	*du39*	*su2*	*su1*
	Gel strength (g)			
1 day at 25 °C	13.6bB	11.2bC	1.0aD	18.2bA
7 day at 4 °C	16.9aC	25.3aB	4.8aD	30.0aA
	Adhesiveness (g s)			
1 day at 25 °C	13.5bB	11.7bB	17.9aA	19.0bA
7 day at 4 °C	19.1aB	29.2aAB	20.5aB	39.5aA

Means of five replicates. Numbers followed by the same lower case letter within each column within the same block are not significantly different. Numbers followed by the same upper case letter within each row are not significantly different at $P<0.05$. The data for *ae25* starch has been omitted from the table because the starch did not form a gel.

After storage for 7 days at 4 °C, starch from the *su1* line had the strongest gel (Table 6) and all starch gels were stronger (except for the *su2* starch gel) than after storage for 1 day at 25 °C. In contrast to the situation after 1 day of storage, the *du39* starch gel was stronger than the wild type starch gel at 7-day storage at 4 °C. The *du39* starch exhibited a high degree of syneresis, which likely explains the greater increased strength of this mutant starch. The results suggest that the *su1* and *du39* mutant starches may be appropriate for use in products where firm gels are desirable, provided syneresis is not a problem.

After 1-day storage at 25 °C, the *su1* and *su2* starch gels were most adhesive, and the wild type and *du39* gels were the least adhesive. The degree of adhesiveness of the gels increased during the 7-day storage, except for the *su2* starch. In general, stronger gels were more adhesive, except for the *su2* mutant starch, which had a very soft gel with a high adhesiveness value at day 1. There was no significant change in either gel strength or adhesiveness between 1 and 7 days of storage of the *su2* starch, thus the gel texture of this starch could be stable under storage in refrigeration (Li & Corke, 1999).

The TA.XT2 gel strength on 1-day storage correlated with DSC values for Peak II gelatinization of T_o ($r=0.98$, $P<0.05$), δH ($r=-0.96$, $P<0.05$), and R ($r=-0.96$, $P<0.05$). It could be reasoned that a firmer gel would result from a more organized AM–lipid complex (greater T_o of Peak II), which would delay T_o, inhibit swelling, and decrease δH. A narrower peak (R) would result from homogeneity in the structures involved in the complex. Gel-strength after 7-day storage positively correlated with the R of Peak II during gelatinization ($r=0.98$, $P<0.05$). The wider the Peak II during gelatinization, the more opportunities for re-organization and perfection of the complex formation.

4. Conclusions

The correlation analyses indicated that the AM percentage and the branch-chain-length profiles of the starch molecules play an important role in the functional properties of the whole starch. Also, correlations established between the thermal and functional properties of starches aid in understanding the potential relationships between the structural features of the starch molecules and their effects on more than one kind of functional behavior. The significant contributions of the starch components suggest the importance of knowing the structural features needed for specific applications.

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