J. Agric. Food Chem. **1999,** *47,* 4939–4943

Physicochemical Properties of Maize Starches Expressing Dull and Sugary-2 Mutants in Different Genetic Backgrounds

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Isogenic lines carrying the *du* or su_2 genes in five different maize inbred lines were developed, and the effect of the starch-modifying genes on general, thermal, pasting, and gel textural properties of starch was studied. Swelling power of *du* and su_2 starches was significantly lower than that of normal starch. The peak viscosity of *du* starches was reduced, peaks were sharper, and the setback was lower than in normal starches. The hardness of *du* starch gels increased and the adhesiveness decreased severalfold during 7 days of storage, indicating unstable gel texture. The su_2/su_2 starches had flatter viscosity peaks than normal starches, with extremely low viscosity and almost zero breakdown and setback. In the su_2 starch gels hardness and adhesiveness did not change significantly during 7 days of storage. For most measured properties, there was considerable variation among different inbred backgrounds carrying the same mutant genotypes. Genetic background and starch-modifying genes can both be manipulated to select specific desired starch properties.

Keywords: *Maize; starch mutants; dull; sugary; pasting properties; gelatinization; gel texture*

INTRODUCTION

Maize starch, an important product of the grain-processing industry, is used in a wide range of applications in the food and nonfood industries. To better suit these uses, the starch may be modified through chemical, physical, or biological (genetic) means (Orthoefer, 1987; Sanders et al., 1990). With the growing interest in healthful and natural foods over the past decade, chemically modified starches are losing their attractiveness for food applications, and maize starches modified by genetic means (which can be labeled as natural or native) are the focus of increased attention. Several known endosperm mutant genes in maize, *ae*, *du*, su_2, and *wx*, termed starch-modifying mutant genes, alter the structure and properties of starches but have little effect on starch quantity (Glover, 1989). These genes are important resources for the commercial development of genetically modified maize starches. A number of patents for genetically modified maize carrying different starch-modifying genes have been granted in recent years [e.g., Katz (1991) and Ng et al. (1997)].

Much work has been done on the starch structure and physicochemical properties of starch-modifying mutants. Inouchi et al. (1984) and Boyer and Liu (1985) found that *du* and su_2 genes are associated with increased amylose content to different extents. On the basis of differential scanning calorimetry (DSC) analysis, the onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy (ΔH) of su_2/su_2 starch were significantly lower than in starches from other mutant genotypes (Inouchi et al., 1991; Li and Glover, 1997). The value of ΔH for *du*/*du* starch was smaller than for normal starch (Brockett et al., 1988). Wang et al. (1993) characterized physicochemical properties of maize starches from 17 mutant genotypes in Oh43 background, although su_2/su_2 was not included and the investigations were done with a very limited genetic background. They found that starch from *du*/*du* had a low viscosity that increased consistently during heating and cooling and had a firmer gel after 7 days of storage. White et al. (1994) described the properties of su_2/su_2 starch, finding an amylose content of 35%, smaller starch granules than normal starch, and suitable pasting properties for application in starch-thickened acidic foodstuffs.

More recently, the effects of genetic background on some starch properties of several maize mutant genes have been noted. Sanders et al. (1990) reported that genetic background influenced the variation in thermal properties and amylopectin fine structures among *wx*-containing genotypes. Significant differences for thermal properties were observed among the su_2 populations from crosses of exotic germplasm with Oh43su_2 (Campbell et al., 1993). However, there is little information about the effects of genetic background on other physicochemical properties. Furthermore, to understand the effects of genetic backgrounds, it is essential to expand these studies to starches with the su_2 mutant in different background lines. China is the world's second largest maize producer, with an output of some 100 million tons per year. Inclusion of major Chinese inbred lines in such studies would add greatly to their usefulness for agricultural development in China.

The purpose of this study was to evaluate the effects of the starch-modifying genes, *du* and su_2, in five different genetic backgrounds, on the thermal, pasting, and gel textural properties of starch.

MATERIALS AND METHODS

Materials. Five sets of isogenic lines with different backgrounds (maize inbreds A632, Oh43, Hz85, Hz101, and Hz47) carrying either *du*/*du* or su_2/su_2 genotype, as well as their

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10.1021/jf990238a CCC: $18.00

normal counterparts, were used. In the Hz47 background only two types were obtained, normal and su_2/su_2. The A632 and Oh43 isogenic lines were kindly provided by Dr. D. V. Glover, Purdue University, West Lafayette, IN. They were developed by backcrossing for at least six generations. All lines were grown in uniform field conditions following normal agronomic practices on the Agronomy Farm at Huazhong Agricultural University, Wuhan, People's Republic of China, in 1997. Starch was extracted as described previously (Li et al., 1994).

Amylose Determination. The method of Blakeney et al. (1994) with modifications was used to measure apparent amylose content (AM). Starch (100 mg) was weighed into a 100-mL flask and mixed with 1 mL of ethanol (95%) and 9 mL of sodium hydroxide (1 M). Samples were heated in boiling water for 15 min. After cooling, distilled water was used to dilute to 100 mL. Then 2.5 mL of dispersed starch solution was pipetted into a 50-mL flask, and 0.5 mL of acetic acid (1 M) and 1 mL of I_2−KI were added. The solution was diluted with distilled water up to 50 mL, mixed, and left to stand for 20 min. The absorbance was read at 620 nm. A standard curve was made with waxy corn and 70% high-amylose corn starches. Triplicate measurements were made on each sample.

Swelling Power and Solubility. Swelling power and solubility were determined following the method of Wang et al. (1993) with slight modifications. Starch (0.6 g, dwb) was mixed with 30 mL of distilled water and heated at 85 °C for 30 min. The clear supernatant was centrifuged at 2000g for 15 min, decanted into a preweighed dish, and dried at 130 °C overnight. Swelling power and solubility were computed as the ratios of the sediment weight to initial starch weight and as a percentage of starch dissolved in water, respectively. Triplicate measurements were made on each sample.

Starch Digestibility. Following the methods of Zhang et al. (1995) and Liu et al. (1997) with modifications, 100 mg (dwb) of starch was weighed into a preweighed tube and mixed with 6 mL of distilled water. The sample was boiled for 15 min. After the mixutre had cooled to room temperature, 50 units of α-amylase (Sigma Chemical Co., St. Louis, MO) was added, and the mixture was then incubated at 37 °C for 20 min or 2 h. Ethanol (6 mL, 90%) was used to precipitate undigested starch. After centrifugation, the starch pellet was oven-dried to constant weight at 80 °C. Starch digestibility was calculated as the percentage weight loss after digestion. Triplicate measurements were made on each sample.

DSC. Thermal properties were evaluated by using a Mettler DSC20 instrument (Mettler, Naenikon-Uster, Switzerland). Following the methods of Li et al. (1994) and Wu et al. (1995), 3.5 mg of starch (dwb) and 8 μL of distilled water were weighed into the DSC pan, mixed, and equilibrated for an hour, and then samples were run from 25 to 125 °C at a rate of 10 °C/min. Gelatinization onset (T_o), conclusion (T_c), and peak temperature (T_p) and enthalpy (ΔH) were recorded. The gelatinization range (T_r) was computed as $T_c - T_o$. Duplicate measurements were made on each sample.

Viscoamylography. A Rapid Visco-Analyser Model 3D (RVA) (Newport Scientific Pty. Ltd., Warriewood, Australia) was used to test pasting properties. Starch (2.5 g, dwb) and distilled water were weighed into an RVA canister to a total weight of 28 g (8.9% starch). A time−temperature profile under constant shear was applied as follows: holding at 50 °C for 2 min, heating to 95 °C in 7.5 min, holding at 95 °C for 5 min, cooling to 50 °C in 7.5 min, and holding at 50 °C for 5 min. The peak viscosity (PV), hot paste (holding) viscosity (HPV), and cool paste (final) viscosity (CPV) were determined. Breakdown (BD) and setback (SB) were calculated as PV − HPV and HPV − CPV, respectively. Duplicate measurements were made on each sample.

Gel Texture. Starch gel texture was measured with a TA.XT2*i* texture analyzer (Texture Technologies Corp., Scarsdale, NY; Stable Micro Systems, Godalming, Surrey, U.K.), equipped with the Texture Expert software program (version 5.16), as described by Liu et al. (1997). After the RVA test, the starch paste was stored at 25 °C for 1 day or at 4 °C for 7 days before texture testing. A cylindrical flat-ended 5 mm probe was used to compress the gel at a speed of 1.0 mm/s for a distance of 10 mm. Hardness (grams) and adhesiveness (gram seconds) of the starch gel were computed from the instrument software. Two gels were prepared for each treatment for each starch sample. Multiple (four to five) repetitions of the texture test on each gel were performed.

Table 1. General Properties of Starch Mutants in Five Different Genetic Backgrounds

source	amylose (%)	swelling power (g/g)	solubility (%)	digestibility	
				20 min	2 h
A632+	26.8	12.8	10.6	87.1	89.0
A632*du*	29.0	7.7	6.8	78.4	80.7
A632su_2	29.5	5.2	12.8	72.8	76.4
Oh43+	24.7	10.3	13.8	84.7	86.6
Oh43*du*	35.7	9.5	15.3	87.4	89.9
Oh43su_2	36.7	7.6	9.1	82.8	84.6
Hz85+	25.8	11.0	9.4	86.8	89.1
Hz85*du*	37.0	9.2	13.4	81.6	84.2
Hz85su_2	43.2	7.1	8.3	82.1	84.5
Hz101+	28.3	9.5	7.5	87.5	89.0
Hz101*du*	37.6	7.9	8.7	84.2	86.7
Hz101su_2	33.7	7.0	6.4	82.8	84.9
Hz47+	26.6	9.9	8.1	87.0	90.0
Hz47su_2	36.4	6.3	7.1	81.2	84.4
LSD[a]	2.08	0.66	0.95	0.95	0.98

[a] Least significant difference ($P < 0.05$) for comparison of means in the same column.

Statistical Analysis. Data were analyzed with the SAS program version 6.04 (SAS Institute, Cary, NC). Least significant differences (LSD) for comparison of means were computed at $P < 0.05$.

RESULTS AND DISCUSSION

General Properties. Both *du*/*du* and su_2/su_2 genotypes in different backgrounds significantly elevated AM (Table 1), which is in agreement with previous reports (Inouchi et al., 1991; Boyer and Liu, 1985), but the level of increase depended on genetic background. When compared in the same genotype (either *du*/*du* or su_2/su_2), AM content varied widely among inbred backgrounds. AM of *du*/*du* and su_2/su_2 starches in A632 was lowest among all backgrounds, whereas those of Hz101*du* (37.6%) and Hz85su_2 (43.2%) were highest for the *du*/*du* and su_2/su_2 genotypes, respectively.

Swelling powers of *du*/*du* and su_2/su_2 starches in all backgrounds were significantly lower than starches from their normal counterparts (Table 1), probably due to the increases in AM contents. However, su_2/su_2 starch had considerably lower swelling power than *du*/*du* starch in the same backgrounds. There was significant variation for swelling power within both *du*/*du* and su_2/su_2 depending on inbred background. Solubility varied from 6.4% (Hz101su_2) to 15.3% (Oh43*du*).

In general, digestibility of the normal starches was higher than both the *du*/*du* and su_2/su_2 starches for the same inbred background, except for Oh43*du*. The su_2/su_2 starches had the lowest digestibility within an inbred background, contrary to the results of Fuwa et al. (1979), who found that the digestibility of su_2/su_2 starch was 2-fold higher than that of normal starch. This may be attributed to methodological differences, as in the present study gelatinized starches were used. However, there were no significant differences for starch digestibility among su_2/su_2 genotypes except for A632su_2.

Thermal Properties. Within any inbred background T_p and ΔH of su_2/su_2 starches were lowest, ranging from 61.3 °C (Hz47su_2) to 63.9 °C (A632su_2) and from 3.4 J/g (A632su_2) to 5.2 J/g (Hz85su_2), respectively. The lowest T_o and T_c were also observed for su_2/su_2. These results

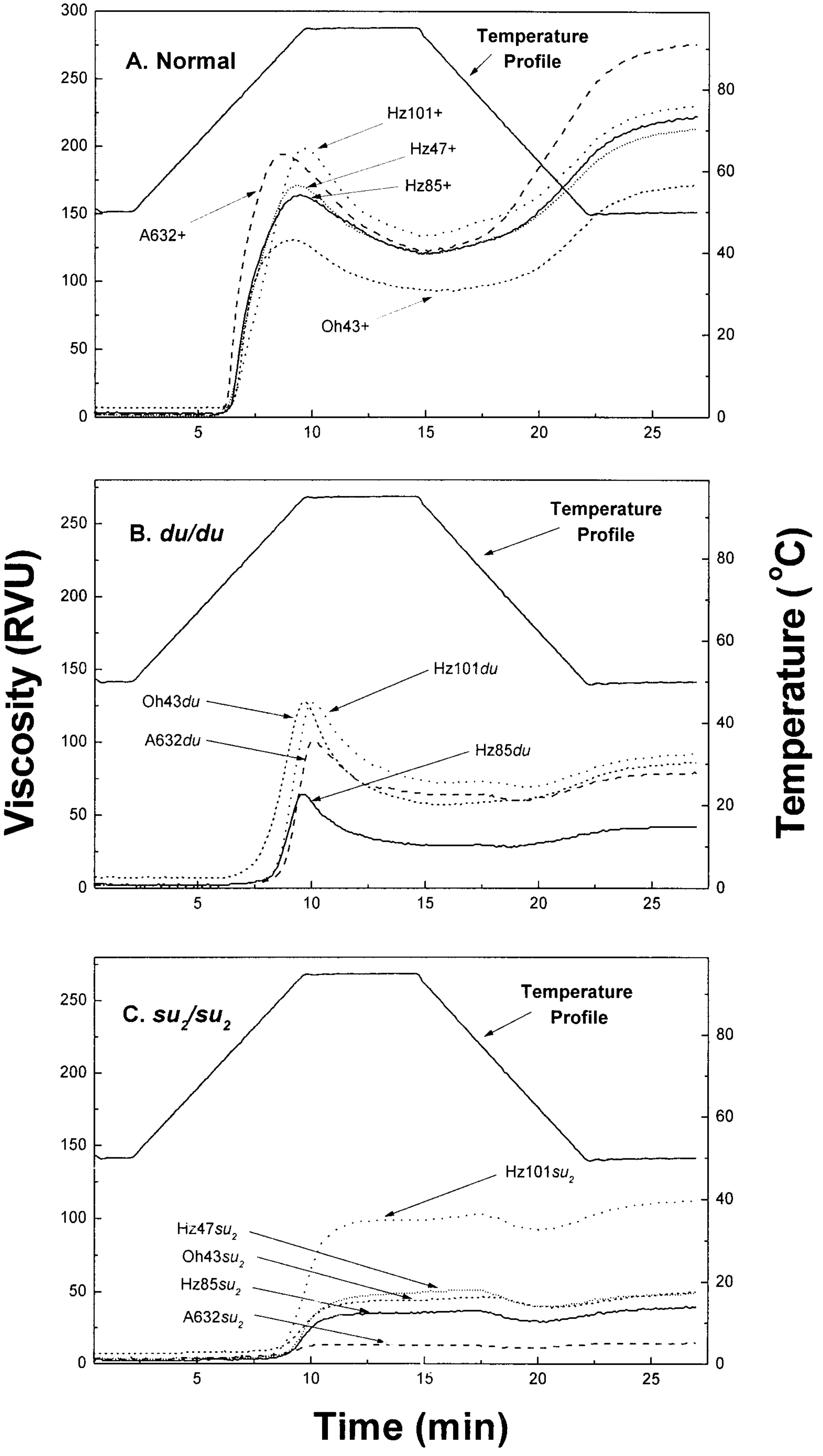

Figure 1. RVA pasting profiles of starch of five inbred line backgrounds carrying (a) normal (nonmutant) starch genes, (b) *du/du*, and (c) su_2/su_2. RVU, rapid viscosity units.

Table 2. Thermal and Pasting Properties of Starch Mutants in Five Different Genetic Backgrounds

source	T_p (°C)	ΔH (J/g)	T_o (°C)	T_c (°C)	T_r (°C)	PV (RVU)	HPV (RVU)	CPV (RVU)	BD (RVU)	SB (RVU)
A632+	72.2	12.0	67.9	78.2	10.3	196	127	281	69	154
A632*du*	72.6	9.1	67.7	79.4	11.7	100	61	77	39	17
A632su_2	63.9	3.4	58.7	70.4	11.8	13	13	14	0	1
Oh43+	75.0	12.6	71.1	81.7	10.7	129	89	165	40	76
Oh43*du*	74.0	12.0	70.4	79.9	9.6	128	57	85	71	28
Oh43su_2	61.8	4.8	57.0	67.5	10.5	45	42	48	3	6
Hz85+	74.0	12.3	69.9	80.5	10.6	162	118	220	45	102
Hz85*du*	74.3	9.1	69.6	79.7	10.1	64	29	41	35	12
Hz85su_2	62.6	5.2	59.0	68.6	9.7	26	36	39	0	3
Hz101+	72.6	11.7	68.6	79.4	10.8	198	133	228	65	95
Hz101*du*	73.1	10.4	69.1	79.4	10.4	127	69	90	59	22
Hz101su_2	61.9	5.1	57.9	67.5	9.6	103	99	112	4	13
Hz47+	72.6	11.8	68.2	79.1	10.9	171	118	210	53	92
Hz47su_2	61.3	4.8	57.6	67.2	9.6	51	49	49	2	0
LSD[a]	0.47	0.63	0.30	0.82	1.01	3.7	6.0	4.7	2.8	3.1

[a] Least significant difference ($P < 0.05$) for comparison of means in the same column.

are similar to those found in limited backgrounds by Inouchi et al. (1991) and Li and Glover (1997). The T_p and ΔH of *du*/*du* starches varied between 72.6 °C (A632*du*) and 74.3 °C (Hz85*du*) and between 9.1 J/g (Hz85*du*) and 12.0 J/g (Oh43*du*), respectively. For normal and *du*/*du*, T_p was significantly different in the Oh43 background, but not in A632, Hz85, and Hz101, whereas ΔH was significantly lower in A632, Hz85, and Hz101 backgrounds but not for Oh43. Both *du*/*du* and su_2/su_2 had similarly high AM contents relative to normal starch, but all measured thermal properties of su_2/su_2 starches were greatly different from those of *du*/*du* starches. This indicated that other factors influence these properties besides AM content. When starches of different inbred backgrounds were compared within the same genotype (*du*/*du* or su_2/su_2), there were significant differences for most of the thermal properties.

Pasting Properties. The RVA curves (Figure 1) show clearly that the starches of the three genotypes, normal, *du*/*du*, and su_2/su_2, exhibited very different pasting properties. As expected, normal starches had high viscosity and setback (SB). However, *du*/*du* starches showed sharp peaks of viscosity with quite low viscosity during heating and a remarkably low SB on cooling, indicating greater resistance to shear thinning, whereas su_2/su_2 starches had flatter peaks with very low viscosity and almost no BD and SB during heating and cooling, which may be caused by highly restricted swelling. These pasting properties of su_2/su_2 starch resembled those of chemically modified cross-linking starches (White et al., 1994).

Numerical parameters for the pasting properties (summarized in Table 2) show that although the basic shapes of pasting curves were similar for lines with the same mutant genotype, there was wide variation for most pasting properties among different backgrounds. For *du*/*du* starch, line Hz101*du* (an inbred line from germplasm of tropical origin) had the second highest PV [127 rapid viscosity units (RVU)] and the highest HPV (69 RVU) and CPV (90 RVU), whereas HZ85*du* had the lowest PV (64 RVU), HPV (29 RVU), and CPV (41 RVU), ∼2-fold differences for these pasting properties. The ranges of BD and SB among four backgrounds were 71−35 and 28−12 RVU, respectively. Among five different inbred background su_2/su_2 genotypes, Hz101su_2 starch showed the highest PV (103 RVU), HPV (99 RVU), and CPV (112 RVU) (similar to the case with *du*/*du*), but A632su_2 had the lowest PV (13 RVU), HPV (13 RVU), and CPV (14 RVU). Because the comparisons were in isogenic lines, these differences in pasting properties could be attributed to the effect of inbred genetic backgrounds, which may modify and regulate the expression of mutant genes during endosperm development so that starch fine structure could change as reported for *wx* isogenic lines (Sanders et al., 1990). The interactions between mutant genes and genetic background are highly complex.

Table 3. Gel Properties of Starch Mutants in Five Different Genetic Backgrounds

	hardness (g)			adhesiveness (g s)		
source	1 day	7 days	7 days/ 1 day	1 day	7 days	7 days/ 1 day
A632+	43	67	1.56	62	51	0.81
A632*du*	18	63	3.45	35	169	4.81
A632su_2	9	9	1.20	17	19	1.12
Oh43+	32	69	2.15	60	64	1.06
Oh43*du*	30	102	3.40	52	165	3.16
Oh43su_2	37	41	1.11	84	77	0.92
Hz85+	28	86	3.09	72	171	2.39
Hz85*du*	28	130	4.59	66	325	4.96
Hz85su_2	42	44	1.04	95	89	0.93
Hz101+	34	65	1.88	67	91	1.35
Hz101*du*	31	101	3.23	76	251	3.31
Hz101su_2	42	49	1.18	79	80	1.02
Hz47+	36	66	1.86	88	119	1.35
Hz47su_2	31	35	1.12	70	95	1.37
LSD[a]	3.8	9.5		11.4	51.8	

[a] Least significant difference ($P < 0.05$) for comparison of means in the same column.

Gel Textural Properties. Gel hardness and adhesiveness of *du*/*du* starches after 1 day of storage were similar to those of normal starches, except in an A632 background (Table 3). Generally, the gel hardness and adhesiveness of both normal and *du*/*du* starches increased during storage. However, the gel hardness of *du*/*du* starches after 7 days of storage became 3.2−4.6 times firmer, and adhesiveness was 3.2−4.5 times more sticky than after 1 day of storage, indicating instability of the *du*/*du* starch gels during refrigerated storage. Syneresis of water from *du*/*du* gels was also observed, in agreement with Wang et al. (1993). A comparison of gel hardness and adhesiveness of su_2/su_2 starches after 1 day of storage showed that some inbred backgrounds were firmer and others softer than the normal, ranging from 9 g (A632su_2) to 42 g (Hz85su_2) and from 17 (A632su_2) to 95 g·s (Hz85su_2), respectively. Neither gel hardness nor adhesiveness changed significantly between 1 and 7 days of storage, showing that the gel texture of su_2/su_2 starches would be quite stable during refrigerated storage. A comparison of the same starch-modifying genotype in different inbred backgrounds revealed significant variation in gel hardness and adhesiveness.

Conclusions. This study provided further evidence that the starch-modifying genes, *du* and su_2, significantly alter the physicochemical properties of starch. The results of RVA clearly showed that the three genotypes, normal, *du*/*du*, and su_2/su_2, had different pasting properties. The gel texture of *du*/*du* starches was unstable during refrigerated storage, whereas that of su_2/su_2 starches was more stable. These novel properties of *du*/*du* and su_2/su_2 starches provide additional opportunities to develop new food and nonfood products with genetically modified starches. Although the starch-modifying genes play a vital role in determining starch properties, genetic background also significantly influences the physicochemical properties. The wide variation of different properties of starches among different inbred backgrounds for the same starch-modifying genes demonstrates a complex pattern of control. Our results on the effect of genetic background on the AM content, pasting properties, and gel texture show that it is necessary to select the suitable inbred backgrounds in maize breeding for precisely targeted genetic modification of starch.

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Received for review March 10, 1999. Revised manuscript received September 13, 1999. Accepted September 19, 1999. Financial support was received from the Hong Kong Research Grants Council and the University of Hong Kong Committee on Research and Conference Grants.

JF990238A