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Behavior of starches derived from varieties of maize containing different genetic mutations: effects of starch genotype on granular morphology

F.R. Katz, S.L. Furcsik, F.L. Tenbarge, R.J. Hauber & R.B. Friedman

American Maize-Products Co., Hammond, Indiana 46320-1094, USA

Starch was isolated from several maize genotypes and surveyed for granular morphology, granular size distribution, apparent amylose content, and thermal properties. Each genotype had a distinct granular morphology. Morphology was classified according to the presence of the *waxy*, *dull*, and *amylose-extender* genes.

INTRODUCTION

An important aspect of natural starch structure is its organization into granules. Morphology of the granule has been shown to reflect the plant species, genotype, background, and conditions present during a particular growing year (Shannon & Garwood, 1984). Maize granules can be grouped by morphology into four categories: generally spherical, generally angular, dimpled, and irregular (Fannon *et al.*, 1992). In maize, the *amylose-extender* (*ae*) gene has been shown to result in both greatly elongated granules and granules with protrusions. The amount of protrusions increased with the *ae* gene dosage. The protrusions are thought to be due to secondary initiation (Boyer *et al.*, 1976). Maize genotypes, such as horny and floury, are thought to affect the protein content and packing within the kernel, rather than the biosynthetic pathway of starch (Glover & Mertz, 1987). This paper will discuss the impact of maize genotype on granule morphology and size distribution. It will be shown that granule morphology is affected not only by genotype deletions which alter the biosynthetic pathway, but also by those genes which are known to alter the protein content and thought to influence the packing in the maize kernel.

EXPERIMENTAL

Starches

Where possible, starches were extracted from hybrid maize plants. Wet milling was performed in a commerical process or by laboratory steeping in a manner analogous to plant scale steeping based on the procedure of Anderson (1963).

Particle size analysis

Analysis was performed using a Brinkmann particle size analyzer model 2010. Data presented here were the results of volume mode calculations. Two milligrams of starch were suspended in 100 ml of deionized water. Aliquots of 2 ml were measured into polystyrene cuvettes (1 cm × 1 cm) for 5 min intervals. Analysis was to 95% confidence level.

Scanning electron microscopy

Starches were examined using a JOEL JSM-840 electron microscope. Starch was sprinkled on double-sided tape and coated with approximately 200 Å of gold before viewing. At least four representative samples were photographed for each starch.

Differential scanning calorimetry

Calorimetry was carried out on starch samples at 30% solids using a Mettler model DSC 30. Slurries were heated at a rate of 10°C/min from 10 to 130°C, in sealed 40 μl aluminum crucibles. An empty crucible was used as the reference cell. Experiments were performed in triplicate; averages reported had a deviation less than 0·1°C and 0·4 J/g.

Table 1. Physical characteristics of starches from different maize genotypes

Maize genotype	Particle size volume mode (μm)	Apparent amylose (%)	DSC endotherm peak temp. (°C)	DSC enthalpy (J/g)
Containing waxy (wx)				
wx	16·4	0	75·1	16·0
aewx	12·1	21	82·9	13·0
duwx	13·1	0	72·0	15·6
wxfl1	17·1	0	71·4	14·1
wxsh1	16·4	0	73·2	15·0
Containing dull (du)				
du	14·0	31	71·1	10·8
duh	15·6	31	71·3	10·3
duwx	13·1	0	72·0	15·6
aedu	10·4	45	70·0	8·0
dusu 2	15·6	46		
aedush1	12·2	51	72·6	10·4
Containing amylose-extender (ae)				
aeae	12·1	50	79·2	13·3
aeaeae	11·5	70	95·9	7·0
aedu	10·4	45	70·0	8·0
aedush1	12·2	51	72·6	10·4
aesu2	12·6	65	77·5	16·5
aewx	12·1	21	82·9	13·0
Control				
common	15·3	25	70·8	10·7
h	19·2	26	72·8	10·9

Apparent amylose

Apparent amylose was determined by iodine binding (blue value) as specified by tentative standard 7-25-75 of the Corn Industries Research Foundation.

RESULTS AND DISCUSSION

Table 1 summarizes the physical characteristics of the maize genotypes surveyed. Because the purification procedure used here was intended to simulate commercial wet milling, the effects of enzymatic hydrolysis can be observed in some granules in these photomicrographs.

Maize starches containing the *waxy* (*wx*) gene

Granules from *dull waxy* (*duwx*) maize (Fig. 1) and *amylose-extender waxy* (*aewx*) (Fig. 2) were oddly shaped, with fewer angular and nearly spherical granules, when compared to those of *waxy* (*wx*) maize (Fig. 3). The size distributions volume mode of starches

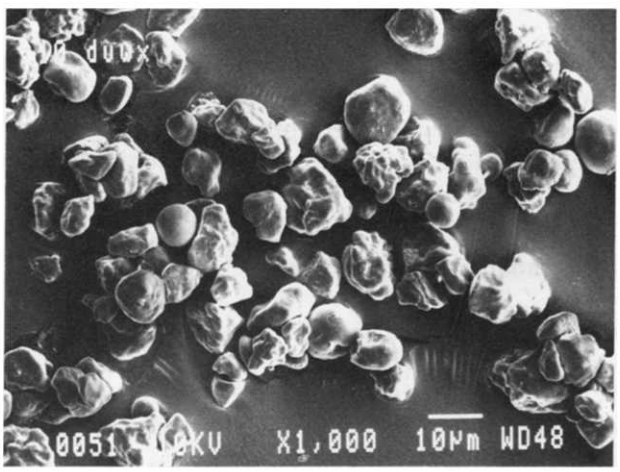

Fig. 1. Scanning Electron Micrograph (SEM) of starch from *duwx* maize starch. (Original mag. ×1000; current mag. ×700.)

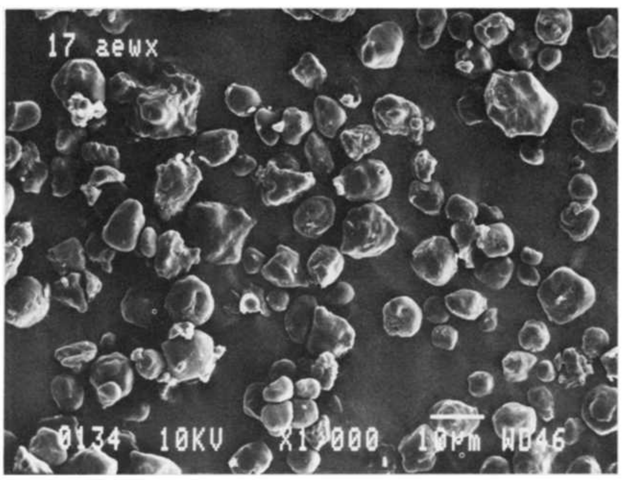

Fig. 2. SEM of *aewx* maize starch. (Current mag. ×700.)

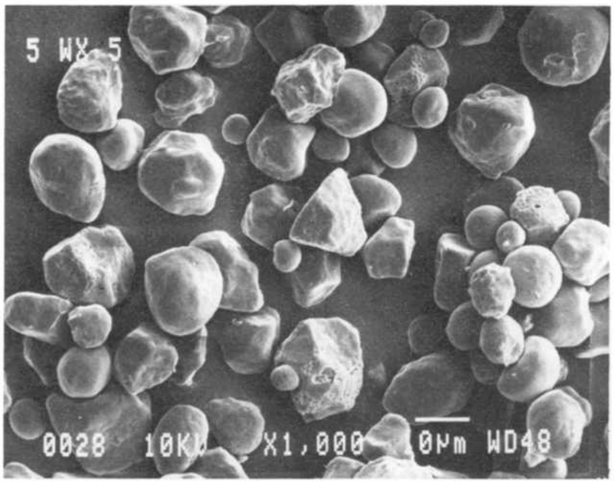

Fig. 3. SEM of *wx* maize starch. (Current mag. ×700.)

from these genotypes were also found to be smaller when compared to *wx* (Table 1).

Granules isolated from the *waxy floury1* (*wxfl1*) genotype showed fewer angular granules when compared to *waxy* (Fig. 4). The size distribution of these granules was greater than that of *wx*; *wxfl1* had a volume mode of 17·1 μm versus 16·4 μm for *wx*. This was attributed to the floury gene which is thought to affect both the protein content of the kernel and the packing of the granules in the endosperm (Glover & Mertz, 1987). The level of protein associated with the purified starch, however, was not changed (Tenbarge *et al.*, unpublished data). The size and morphology of *waxy shrunken1* (*wxsh1*) granules were found to be similar to those of *wx* (Fig. 5, Table 1).

Maize starches containing the *dull* (*du*) gene maize

Granules from *dull horny* (*duh*) were quite large when compared to *du* (Figs 6 and 7). The large size of *duh* granules, when compared to *du* or common, was con-

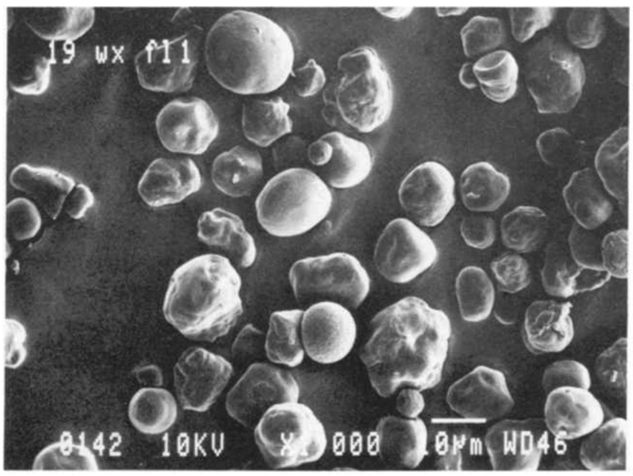

Fig. 4. SEM of *wxfl1* maize starch. (Current mag. ×700.)

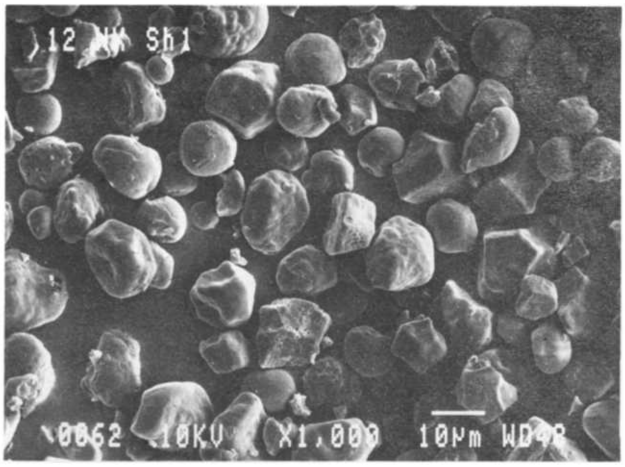

Fig. 5. SEM of *wxsh1* maize starch. (Current mag. ×700.)

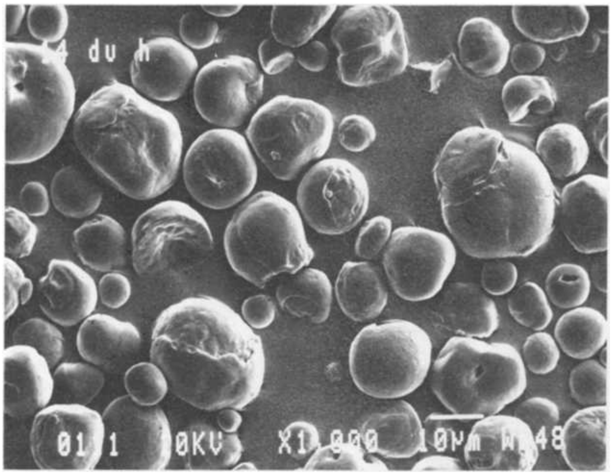

Fig. 6. SEM of *duh* maize starch. (Current mag. ×700.)

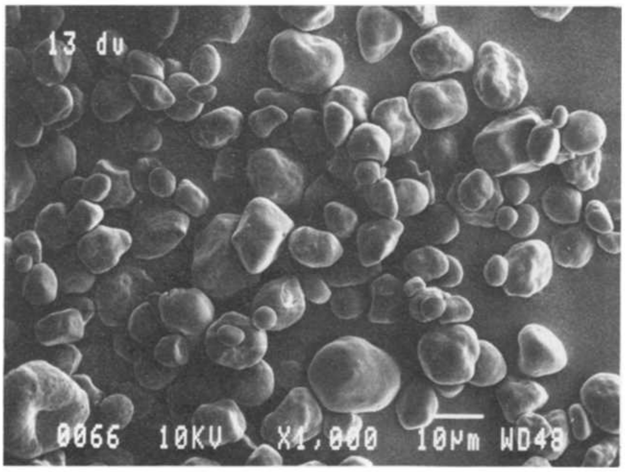

Fig. 7. SEM of *du* maize starch. (Current mag. ×700.)

firmed by particle size analysis (Table 1). Granules of starch from *horny* (*h*) maize were much larger than common. The volume mode found by particle size analysis was 19·2 μm, the largest genotype sampled (Table 1). The *horny* gene is thought to affect the com-

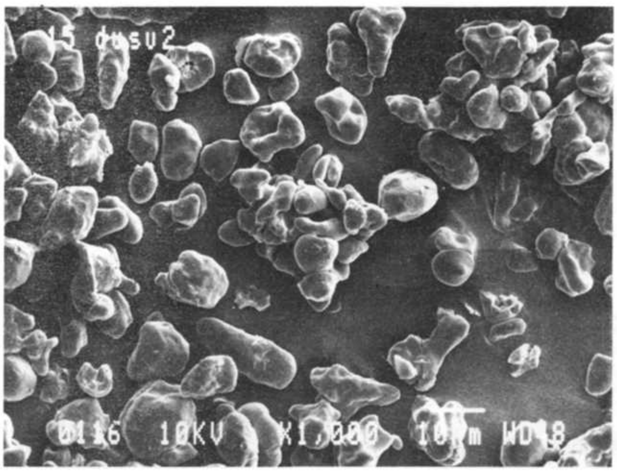

Fig. 8. SEM of *dusu2* maize starch. (Current mag. ×700.)

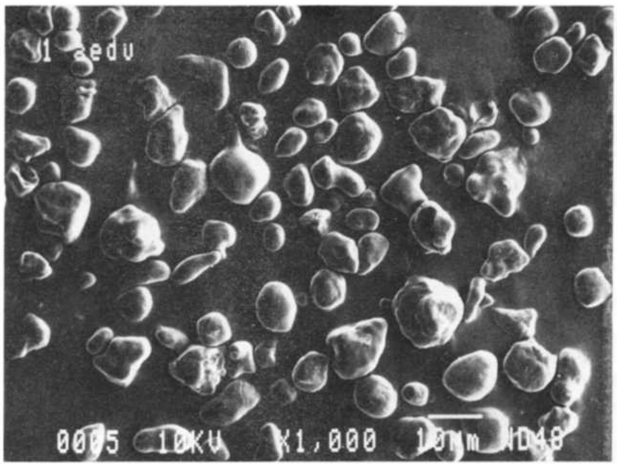

Fig. 9. SEM of *aedu* maize starch. (Current mag. ×700.)

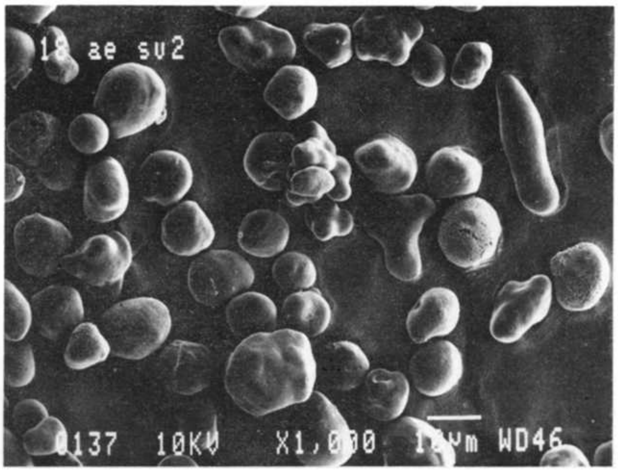

Fig. 10. SEM of *aesu2* maize starch. (Current mag. ×700.)

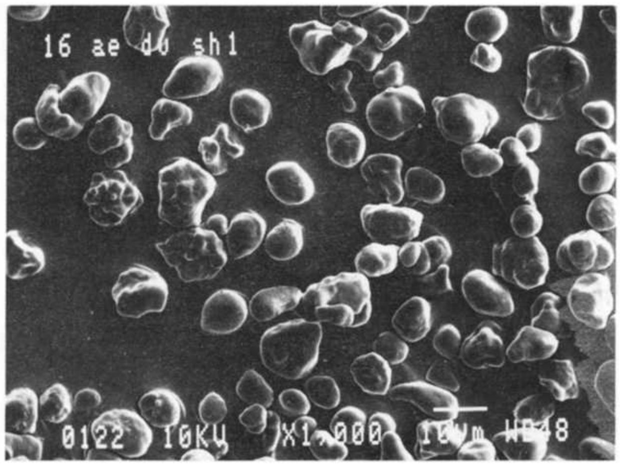

Fig. 11. SEM of *aedush1* maize starch. (Current mag. ×700.)

position of protein in the endosperm, resulting in a 'looser' packing, and, consequently, a larger granule size (Glover & Mertz, 1987).

Starch from *dull sugary2* (*dusu2*) maize had a very unusual morphology. There are some elongated granules as well as granules with protrusions similar to *ae* varieties (Fig. 8).

Maize starches containing the *amylose extender* (ae) gene

Amylose-extender dull (*aedu*) is an example of a genotype producing more linear starches with characteristic morphology (Fig. 9). Characteristic *ae* morphology refers to the elongated granules and granules with protrusions found in amylose-extender genotypes. Particle size analysis of *aedu* shows that its granule size is even smaller than that of *ae*; the average volume mode distribution for *aedu* was reduced to 10·4 μm as compared to 12·1 μm for *ae* genotypes.

Both *amylose-extender sugary2* (*aesu2*) and *amylose-extender dull shrunken1* (*aedush1*) contained, to some degree, elongated irregular granules and protrusions typical of other *ae* maize genotypes (Figs 10 and 11). Particle size analysis showed that this variety's average granule size was also similar to that of *ae*. All had volume modes near 12·0 μm.

All the *ae*-containing starch genotypes had reduced granule size when compared to common or to *wx* maize starches. The volume mode distributions fell close to 12 μm.

ACKNOWLEDGMENT

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