Some Properties of Starches of Opaque-2, Sugary-2
Opaque-2, and Waxy Opaque-2 Mutants of Two
Broad-Based Synthetic Cultivars of Maize

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Summary Starches of the opaque-2, sugary-2 opaque-2 and waxy
opaque-2 endosperm mutants of two broad-based synthetic cultivars
(Temp HA and Temp HB) of maize adapted to the more temperate areas
of the world are, in general, similar to the respective starches of inbred
maize background with regard to amylose percentage and the distribution
of linear α-D-(1→4) linked unit-chains of amylpectin, starch-granule
susceptibility to amylases, and properties examined by X-ray diffracto-
metry and photopastegraphy. Changes in starch content and morphology
of starch granules by scanning electron microscopy in dissected endo-
sperms of the two broad-based synthetic maize cultivars following germi-
nation 0, 2, 4 and 6 days after planting were also investigated.

Key Words starch properties, maize mutants, maize cultivars adapted to
temperate area, opaque-2 maize, sugary-2 opaque-2 maize, waxy opaque-2
maize

Since the discovery of opaque-2 (o2) maize (Zea mays L.) as a high-lysine
cereal (1), the urgent need for cultivars adapted to the more temperate areas of
the world has been recognized at Purdue and the International Maize and Wheat
Improvement Center, El Batan, Mexico. Two broad-based synthetics, temperate
(Temp) HA o2 and Temp HB o2 were developed at Purdue from diverse germplasm
from around the world and the U. S. Cornbelt (2).

Furthermore, considerable effort has been concentrated on the development
of double-mutant combinations with o2, sugary-2 opaque-2 (su2o2), waxy opaque-2 (wx

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o_2) and brittle-2 opaque-2 (bt2o_2) (3). The protein quality is at least maintained or is even superior and kernel vitreousness is improved in Temp HA su_2o_2 and Temp HB su_2o_2 cultivars compared to the o_2 synthetics. The wx o_2 double mutant has a protein quality equivalent to o_2 and is a modified starch type. All of these improved-quality maize cultivars, however, have reduced agronomic yield when compared to the more elite normal hybrids, and from this standpoint their acceptance has been limited.

This paper describes the isolation and characterization of maize starch granules from kernels of Temp HA o_2, Temp HB o_2, Temp HA su_2o_2, Temp HB su_2o_2, and Temp HB wx o_2 broad-based synthetics. Inbred lines Oh43 su_2o_2, Oh43 wx o_2, Oh43 ae, B37 ae near isogenic conversions and the Oh43 and B37 normal counterparts were used as controls in some characterizations for comparison. The properties of the starches of these improved high-quality protein maize synthetics have not heretofore been investigated. Amylose percentage and the distribution of average chain-lengths of amylopectin were determined by the use of gel filtration after debranching starch with Pseudomonas isoamylase. Some physical and chemical properties of the starches were examined by scanning electron microscopy (SEM), X-ray diffractometry and photopastegraphy, together with evaluation of starch-granule susceptibility to amylases. Changes in starch content and morphology by SEM of starch granules in dissected endosperm of maize cultivars adapted to the temperate areas following germination 0, 2, 4 and 6 days after planting were also investigated.

**MATERIALS AND METHODS**

**Maize kernels.** Mature maize (Zea mays L.) kernels of several endosperm mutants in two broad-based synthetic backgrounds were used: Temperate (Temp) HA o_2, Temp HB o_2, Temp HA su_2o_2, Temp HB su_2o_2 and Temp HB wx o_2. In addition, inbred lines Oh43 su_2o_2, Oh43 wx o_2, Oh43 ae, B37 ae near isogenic conversions and the Oh43 and B37 normal counterparts were used as controls in some characterizations for comparison. The materials were grown at the Purdue Agronomy farm.

**Starch granules.** Starch granules were prepared by a method reported previously (4).

**Sources of enzymes.** Crystalline Pseudomonas isoamylase [EC 3.2.1.68] was obtained from Hayashibara Biochemical Laboratories, Inc., Okayama, Japan. Crystalline Rhizopus niveus glucoamylase [EC 3.2.1.3] was purchased from Seikagaku Kogyo Co., Ltd., Tokyo, Japan. Sources of pancreatin, glucose oxidase and peroxidase have been described earlier (4).

**Other methods.** The methods for debranching of starches with Pseudomonas isoamylase, gel filtration of debranched starch on a column of Sephadex G-75, analytical methods for fractionated materials (5), and the methods for determination of starch-granule susceptibility to amylases (4) have been reported pre-
Previously. Preparation of germinating seeds, procedures for specimen mounting and observations of starch granules in dissected maize kernels by SEM have been described earlier (6, 7). Starch contents of maize kernels were determined by a method reported previously (8, 9). X-ray diffractometry was recorded by a method of Hizukuri et al. (10), and the procedure for photopastegraphy has been described earlier (11).

RESULTS

Elution profiles of debranched starches by gel filtration and average chain-lengths of apices of peaks II and III

Table 1 summarizes the distribution of carbohydrate in each fraction (Fr.) and average chain-lengths (CL) of apices of peaks II and III for isoamylase-debranched maize starches of several genotypes of Temp HA, Temp HB, inbred Oh43, and the normal Oh43 counterpart.

Fraction I appeared near the void volume of a Sephadex G-75 column in the normal maize which had typical amylose and amylopectin. This fraction corresponded to amylose of the original starches because the wavelength of maximum absorption ($\lambda_{max}$) of iodine-carbohydrate complexes of the fraction were in a range of longer wavelength (620-640 nm). Fractions II and III originated from amylopectin of the original starches because the degree of polymerization (CL) of their peaks was 40-45 and 15-20 glucose units, respectively. Starches of wx o$_2$, which consist only of amylopectin, had no Fr. I but Frs. II and III. This finding also indicated Fr. I originated from amylose and Frs. II and III from amylopectin (12).

Contents of Fr. I were 23-24% in the normal and o$_2$, 39-41% in su$_2$o$_2$ and 0%.

Table 1. Some properties of isoamylase-debranched components of maize starches of several genotypes of two synthetic cultivars and the inbred Oh43.*

| Fr. I (%) | Intermediate Fr. (%) | Fr. II (%) | Fr. III (%) | Fr. III/II | Peak of Fr. II (CL) | Peak of Fr. III (CL) |
|----------|----------------------|------------|-------------|-----------|--------------------|--------------------|
| Oh43 +   | 23.9                 | 1.6        | 18.9        | 55.6      | 2.9                | 41                 | 16                 |
| Temp HA  o$_2$ | 23.3                | 1.5        | 17.5        | 57.7      | 3.3                | 42                 | 15                 |
| Temp HB  o$_2$ | 24.0                | 1.3        | 17.8        | 56.9      | 3.2                | 40                 | 15                 |
| Oh43 su$_2$o$_2$ | 39.2               | 3.0        | 13.1        | 44.7      | 3.4                | 40                 | 15                 |
| Temp HA  su$_2$o$_2$ | 39.1              | 3.0        | 13.9        | 44.0      | 3.2                | 40                 | 15                 |
| Temp HB  su$_2$o$_2$ | 41.1              | 2.0        | 13.5        | 43.4      | 3.2                | 40                 | 15                 |
| Oh43 wx o$_2$ | 0                   | 2.9        | 26.1        | 71.0      | 2.7                | 41                 | 15                 |
| Temp HB  wx o$_2$ | 0                   | 1.7        | 29.0        | 69.3      | 2.4                | 41                 | 16                 |

*Each fraction (Fr.) was divided according to $\lambda_{max}$ of the carbohydrate-iodine complex as follows: Fr. I, $\lambda_{max}$ ≥ 620 nm, Intermediate Fr., 620 nm > $\lambda_{max}$ ≥ 600 nm, Fr. II, 600 nm > $\lambda_{max}$ ≥ 540 nm, Fr. III, 540 nm > $\lambda_{max}$.
Fig. 1. X-ray diffraction patterns of several maize starches. a, Oh43 normal, b, Temp HB su2o2, c, Temp HA su2o2, and d, Oh43 ae.

Fig. 2. Photopastographs of several maize starches. a, Oh43 normal, b, B37 normal, c, B37 ae, d, Temp HA o2, e, Temp HB o2, f, Temp HA su2o2, g, Temp HB su2o2, and h, Temp HB wx o2. Photopastograms were recorded by the method of Kainuma et al. (24) using a Hirama photopastograph on 0.1% starch suspension at 372 nm.

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in \( wx \ o_2 \). Contents of the intermediate Fr., the ratios of Fr. III to Fr. II, which showed one of the structural characteristics of amylopectin (12), and the chain-lengths of the peaks of both Frs. II and III were similar in the normal, \( o_2 \), \( su_2 o_2 \), and \( wx \ o_2 \) starches examined.

**X-ray diffraction patterns of starches**

Figure 1 shows X-ray diffraction patterns of native starches of Oh43 normal, Oh43 \( ae \), Temp HA \( su_2 o_2 \) and Temp HB \( su_2 o_2 \). The patterns of the native \( su_2 o_2 \) starches are similar to the normal which corresponds to an A-type crystalline

Table 2. Susceptibility to amylases of maize starch granules of several genotypes of two synthetic cultivars and inbred Oh43.

| Maize genotype | Pancreatin | Glucoamylase* |
|----------------|------------|---------------|
|                | % degradation | Relative susceptibility | % degradation | Relative susceptibility |
| Oh43 normal    | 21.5       | 100           | 14.7         | 100           |
| Temp HA \( o_2 \) | 27.3       | 127           | 17.1         | 116           |
| Temp HB \( o_2 \) | 26.5       | 123           | 18.0         | 122           |
| Temp HA \( su_2 o_2 \) | 58.7       | 273           | 30.0         | 204           |
| Temp HB \( su_2 o_2 \) | 55.6       | 259           | 28.8         | 196           |
| Temp HB \( wx \ o_2 \) | 34.5       | 160           | 69.6         | 473           |

*Crystalline preparation from *Rhizopus niveus*. \(^b\) Data were expressed relative to enzyme degradation percentage of the normal Oh43 maize starch, namely % degradation of the normal Oh43 maize starch equals 100 (4).

Fig. 3. Changes in starch content of germinating kernels of several endosperm mutants of two broad-based synthetic maize cultivars. a, Temp HA \( o_2 \), b, Temp HB \( o_2 \), c, Temp HA \( su_2 o_2 \), d, Temp HB \( su_2 o_2 \), and e, Temp HB \( wx \ o_2 \).
structure, but were less crystalline. Not shown were the X-ray diffraction patterns of native starches obtained from o2 and wx o2. The patterns of these starches were very similar to each other and to the normal and corresponded to an A-type crystalline structure which is typical of native starches usually found in cereals.

*Photospectograms of starches*

The normal and o2 starches showed two-step gelatinization curves, which are typical of normal-type starches of cereals (Fig. 2, curves a, b, d and e), while the wx o2 starch had a one-step gelatinization curve followed by a downward slope in a range of higher temperature, which is typical of waxy-type starches of cereals (Fig. 2, curve h). The su2o2 starches are gelatinized to a lesser degree (Fig. 2, curves f, g) and show gelatinization curves which are very similar to that of the ae starches having 31% amylose and 15% intermediate fraction (Fig. 2, curve c).

*Starch-granule susceptibility to amylases*

Table 2 shows values of susceptibility of starch granules for the broad-based synthetic maize cultivars and the normal inbred Oh43 to either pancreatin or crystalline glucoamylase of *R. niveus*. The susceptibility values for o2 starches were nearly identical to those of the normal. The su2o2 starches were digested 2.6-2.7 and 2 times as fast as the normal starch by pancreatin and glucoamylase, respectively. The values for wx o2 starch granules showed quite different susceptibilities. The value for the glucoamylase hydrolysis was the highest among the starches examined.

*Changes in starch contents of germinating kernels of several endosperm mutants of Temp HA and Temp HB cultivars*

Figure 3 shows the time courses of changes in starch contents of germinating kernels 0, 2, 4 and 6 days after planting. Temp HA su2o2 c, showed the greatest decrease in starch content, from 1.50 to 0.182 g/10 kernels (88%) during the six-day period. This was followed by Temp HA o2, a, 1.74 to 0.518 (70%); Temp HB wx o2, e, 1.80 to 0.645 (64%); Temp HB su2o2, d, 1.61 to 0.612 (62%); and Temp HB o2, b, 1.76 to 0.764 (57%).

*SEM of endosperm starches of Temp HB o2 and Temp HB su2o2 germinating kernels*

Enzymatic erosion of starch granules was visible in the dissected endosperm of all of the maize genotypes in the Temp HA and Temp HB backgrounds following germination 4 and 6 days after planting. However, for brevity, we show photoelectronmicrographs of starch granules of the Temp HB o2 and Temp HB su2o2 mutants only (Figs. 4 and 5).

Almost none of the starch granules were attacked by enzymes in Temp HB o2.

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Fig. 4. Scanning electron photomicrographs (SEM) of dissected endosperms of germinating Temp HB o2 maize. a, 2, b, 4, and c, 6 days after planting.

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endosperm 2 days after planting (Fig. 4a). Many starch granules with pores were observed in Temp HB $o_2$ endosperm 4 days after planting (Fig. 4b). The pores on the surfaces of starch granules were bigger in Temp HB $o_2$ endosperm 6 days after planting and sometimes the inner structure of a granule could be observed (Fig. 4c).

A few granules seemed to be attacked in Temp HB $su_2o_2$ endosperm 2 days after planting (Fig. 5a). We were able to observe many starch granules attacked in Temp HB $su_2o_2$ endosperm 4 days after planting (Fig. 5b). Almost all starch granules were attacked in Temp HB $su_2o_2$ endosperm 6 days after planting and the inside step-shaped structures of starch granules were frequently observed (Figs. 5c–e).

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DISCUSSION

These studies showed that the Temp HA \( o_2 \) and Temp HB \( o_2 \) starch properties were very similar to normal (Tables 1 and 2). The results confirm our previous findings for the properties of this mutant in inbred backgrounds with regard to amylose percentage, distribution of linear \( \alpha-(1\rightarrow4) \) linked unit-chains of amylopectin (13) and starch-granule susceptibility to amylases (4, 14). Thus far, we have not observed any major effect of the \( o_2 \) mutant on the properties of starches with the exception of the double mutant \( su_1 o_2 \) where there was a decrease in amylose percentage (15, 16). The Temp HB \( wx o_2 \) starches also showed typical amylopectin properties (12, 13) and were digested faster than normal starches (Tables 1 and 2) as we have shown previously (4, 14) in inbred backgrounds.

The Temp HA \( su_2 o_2 \) and Temp HB \( su_2 o_2 \) starches prepared from mature kernels showed rates of susceptibility to amylase hydrolysis 2 to 3 times higher than normal (Table 2), even though amylose values are higher (4, 12). The X-ray diffractograms showed that the starches of Temp HA \( su_2 o_2 \) and Temp HB \( su_2 o_2 \) were less crystalline than normal in nature, though they were of the A-type found normally. Creech (17) reported X-ray diffraction patterns for 18 and 24 days post-pollination \( su_2 \) kernels to be of the A-type pattern. However, this was in conflict with the observation reported by Dvonch et al. (18) and Badenhuizen (19) who showed mature kernels of \( su \) were of B-type patterns. Creech (17) suggested that the \( su_2 \) granules may change from A- to B-type during development of the kernel to maturity. We showed that \( su_2 o_2 \) starch granules prepared from mature kernels of a 3-way hybrid (Oh43 \( \times \) B37) \( \times \) C103 produced a less crystalline A-type diagram, but following degradation by glucoamylase the residual \( su_2 o_2 \) starch granules produced a B-type diagram (20). Further investigations will be required to characterize the nature of the starch granules of \( su_2 \) and \( su_2 o_2 \) mutants.

Higher gelatinization temperatures have been reported for the high-amylose maize starches (21) and they were incompletely gelatinized even by autoclaving (3 hr at 124°C) (18). The pasting behavior of amylo maize starch measured by a differential scanning calorimetry (22) or in a “Brabender” viscoamylography (23) completely differed from that of the normal and waxy maize starches. Kainuma et al. (24) showed that there is a good correlation between transmittance (372 nm) at 95°C of a photopastegram and swelling power of starch granules at 80°C (25). Our data from photopastegraphy (Fig. 2) showed that some gelatinization occurred for the high-amylose starches of Temp HA \( su_2 o_2 \) and Temp HB \( su_2 o_2 \) as well as those of B37 \( ae \) in the temperature range of 60–100°C; however, the degree of swelling of such starch granules was much less than that of the normal, \( o_2 \) and \( wx o_2 \) starches.

The morphological changes of starch granules as observed by SEM (Figs. 4 and 5) of germinating kernels of the endosperm mutants in the Temp HA and Temp HB backgrounds were similar to those observed in comparable studies of these endosperm mutants in inbred backgrounds (6).
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