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Kernel Composition, Starch Structure, and Enzyme Digestibility of *opaque-2* Maize and Quality Protein Maize

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Objectives of this study were to understand how *opaque-2* (*o2*) mutation and quality protein maize (QPM) affect maize kernel composition and starch structure, property, and enzyme digestibility. Kernels of *o2* maize contained less protein (9.6−12.5%) than those of the wild-type (WT) counterparts (12.7−13.3%). Kernels of a severe *o2* mutant B46*o2* also contained less starch (66.9%) than those of B46wt (73.0%). B46*o2* and QPM starches contained less amylose (28.0 and 26.0%, respectively) than others (31.9−33.7%). The B46*o2* starch also consisted of amylopectin with the fewest branch chains of DP 13−24. Thus, the B46*o2* starch was the most susceptible to porcine pancreatic α-amylase (PPA) hydrolysis. Starches of the dry-ground *o2* maize and QPM were hydrolyzed faster than that of the dry-ground WT maize, resulting from the reduced protein content of the *o2*-maize kernels and the reduced amylose content of the B46*o2* and QPM starch. Starch in the dry-ground maize sample was hydrolyzed faster by PPA (85−91%) than was the isolated starch (62−71%), which could be attributed to the presence of mechanically damaged starch granules and endogenous amylases in the dry-ground maize samples. These results showed that *o2* maize and QPM had highly digestible starch and could be desirable for feed and ethanol production.

KEYWORDS: *Opaque-2* maize; quality protein maize; dry-ground maize; starch digestibility; physicochemical properties

INTRODUCTION

Maize (*Zea mays* L.) is an important crop for food, feed, and fuel applications. Zein is the major protein of maize; however, it is deficient in essential amino acids, tryptophan and lysine. Lysine deficiency in humans and animals causes symptoms of growth impairment, anemia, hypoproteinemia, fatty liver, etc. (*1, 2*).

A mutant called *opaque-2* (*o2*) contains an increased amount of lysine and has been used to improve the protein quality of maize (*3*). The *o2*-maize mutants are known to contain less α-, β-, γ-, and δ-zein, which results in increases in the contents of other proteins, including globulin and albumin (*3*−*5*). The lysine content of *o2* maize can be up to twice that of normal maize. Despite the improvement in the amino acid composition and nutrition value, most *o2*-maize lines are not well-adapted because of their low grain yields, soft and chalky endosperm, and high susceptibility to kernel breakage and to pest and mold damages (*6*).

Quality protein maize (QPM) was developed using modifier genes to produce a vitreous endosperm while maintaining the nutritional advantages of *o2* maize (*6*). The vitreous endosperm, containing an elevated level of γ-zein, increases the resistance of *o2* maize to diseases and insect damages, increases the grain yield, and improves grain appearance (*7*).

The physicochemical properties of starch of *o2* maize and QPM, however, are not well understood. It has been reported that the activities of soluble starch synthase in *o2* maize are terminated earlier than the normal counterpart, resulting in a decrease in starch accumulation (*8*). The activity of granular-bound starch synthase in *o2* maize is also lower than that in the normal counterpart. Starch granules in the *o2*-maize endosperm are synthesized less vigorously than in the normal-maize counterpart. Thus, they are packed more loosely, giving *o2*-maize kernels an opaque appearance (*9*). Starches of single and double mutant *o2*-maize kernels have been shown to be digested more rapidly by α-amylases than that of the normal maize (*10*).

The objectives of this study were to understand how the *o2* mutation and QPM affect the kernel composition, the starch physicochemical properties, and the starch digestibility. This

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Table 1. Kernel Compositions of WT, *o2*, and QPM Maize (db)[a]

lines	%		
	starch[b]	protein[c]	lipid[d]
B46wt	73.0 ± 0.4 b	13.3 ± 0.0 d	4.3 ± 0.2 a
B46o2	66.9 ± 1.4 a	12.5 ± 0.0 bc	5.1 ± 0.1 b
M14wt	71.1 ± 0.8 b	12.7 ± 0.0 c	4.5 ± 0.1 a
M14o2	74.1 ± 0.8 b	9.6 ± 0.0 a	4.2 ± 0.2 a
QPM	72.4 ± 1.3 b	12.1 ± 0.3 b	4.2 ± 0.1 a

[a] Values with the same letter in the same column are not significantly different at $p < 0.05$. [b] Starch contents were determined using Megazyme Total Starch assay kit following AACC Method 76-13 (*14*). [c] Protein contents were determined using macro Kjeldhal method (*13*). [d] Lipid contents were determined using AACC Method 30-25 (*14*).

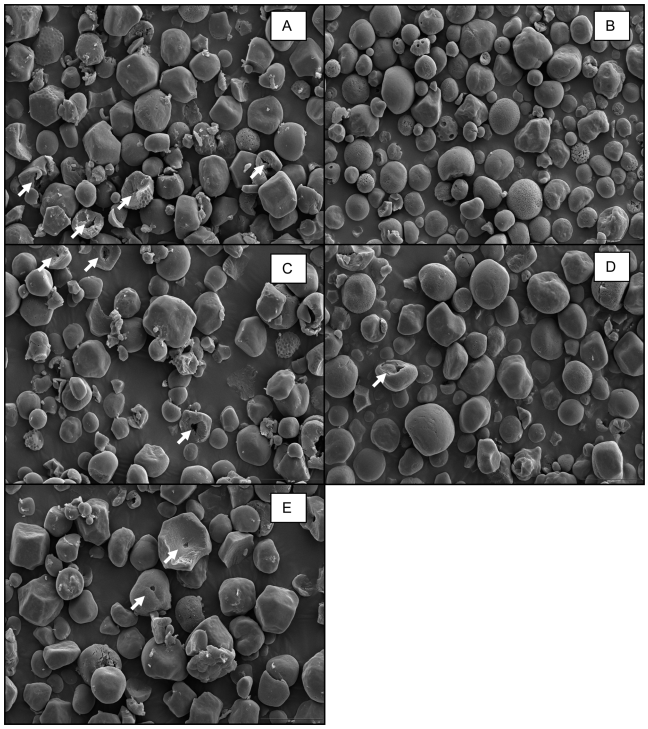

Figure 1. Scanning electron micrographs of starch granules isolated from dry-ground maize of (**A**) B46wt, (**B**) B46*o2*, (**C**) M14wt, (**D**) M14*o2*, and (**E**) QPM. Arrows mark broken starch granules with exposed hila.

information is needed for future breeding and selecting quality *o2* maize and QPM for food, feed, and ethanol production. In this study, we analyzed kernel compositions, starch structures and properties, and starch digestibility of dry-ground wild-type (WT) maize, *o2* maize, and QPM. The enzyme digestibility of starch in the dry-ground maize sample was also compared with that of the isolated starch obtained from wet milling of the whole kernels. Two sets of *o2* maize inbred lines, B46*o2* (severe phenotype) and M14*o2* (mild phenotype), their near-isogenic WT counterparts, B46wt and M14wt, and a QPM sample were selected for this study. The set of near-isogenic lines in the B46 genetic background was selected because the B46*o2* mutant exhibits an extreme phenotype, including the highest lysine content, the lowest kernel density, and the lowest warm germination percentage among eight genetic lines studied by Jia et al. (*11*). The other set of near-isogenic lines in the M14 genetic background was selected for its mild phenotype among the eight genetic lines (*11*).

MATERIALS AND METHODS

Materials. Five maize inbred lines, B46wt, B46*o2*, M14wt, M14*o2*, and a germplasm source known to be a QPM inbred, used in this study were grown at the Iowa State University Agronomy Farm (Boone, IA) in 2004. A single ear of each genotype was produced by self-pollination. The ears were dried and shelled by hand. The dry-ground maize was prepared by grinding whole kernels using a Cyclone Mill (UDY Corp., Fort Collins, CO) to pass through a 0.5 mm screen.

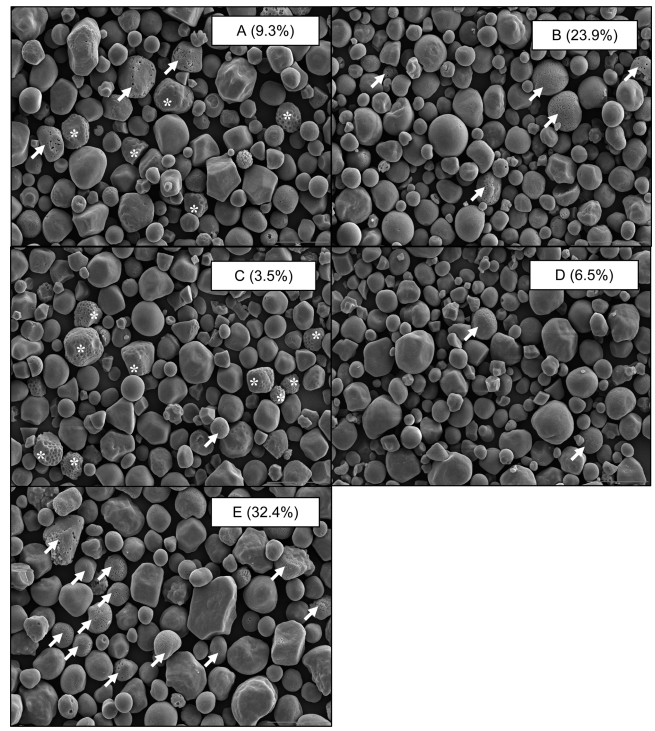

Figure 2. Scanning electron micrographs of starch granules isolated by wet-milling from whole kernels of (**A**) B46wt, (**B**) B46*o2*, (**C**) M14wt, (**D**) M14*o2*, and (**E**) QPM. Numbers in parentheses are the percentages of granules with pinholes. Arrows mark starch granules with pinholes, and asterisks mark starch granules with dimplelike indentations.

Porcine pancreatic α-amylase (PPA), maltohexaose, and maltoheptaose were purchased from Sigma Chemical Co. (St. Louis, MO). Glucoamylase (GA) from *Aspergillus niger*, D-Glucose assay (GOPOD) kits, and Total Starch assay kits were purchased from Megazyme International Ireland Ltd. (Co. Wicklow, Ireland). Crystalline *Pseudomonas* isoamylase was purchased from Hayashibara Shoji, Inc. (Okoyama, Japan), which had a specific activity of about 66000 units/mg protein.

Starch Isolation. Starch was isolated using a wet-milling method (*12*). Samples of maize whole kernels and dry-ground maize (50 g) were steeped in a sodium metabisulfite solution (150 mL, 0.45% w/v) overnight in a refrigerator before blending. For the whole kernels, pericarps and germs were manually removed before the endosperms were blended. The starch isolated from the steeped endosperms was designated as "isolated starch".

Kernel Composition. The nitrogen content of the dry-ground maize was determined using a macro-Kjeldahl method (*13*). The protein content was calculated by multiplying the nitrogen content with 6.25. Lipids were extracted from the dry-ground maize using hexanes in a Soxhlet extractor for 24 h and quantified following AACC Method 30-25 (*14*). The starch content of the dry-ground maize was determined using Megazyme Total Starch assay kit following AACC Method 76-13 (*14*). The protein, lipid, and starch contents were determined in duplicate.

Starch Granule Morphology. The morphology of starch granules isolated from whole kernels (isolated starch) and that of starch granules isolated from the dry-ground maize were examined using a scanning electron microscope (SEM) (JEOL JSM-5800LV, Tokyo, Japan) at the Bessey Microscopy Facility, Iowa State University (Ames, IA), following the method of Jane et al. (*15*).

Starch Digestibility. Starch (100 mg, dry basis, db) or a dry-ground maize sample containing 100 mg of starch (db) was suspended in

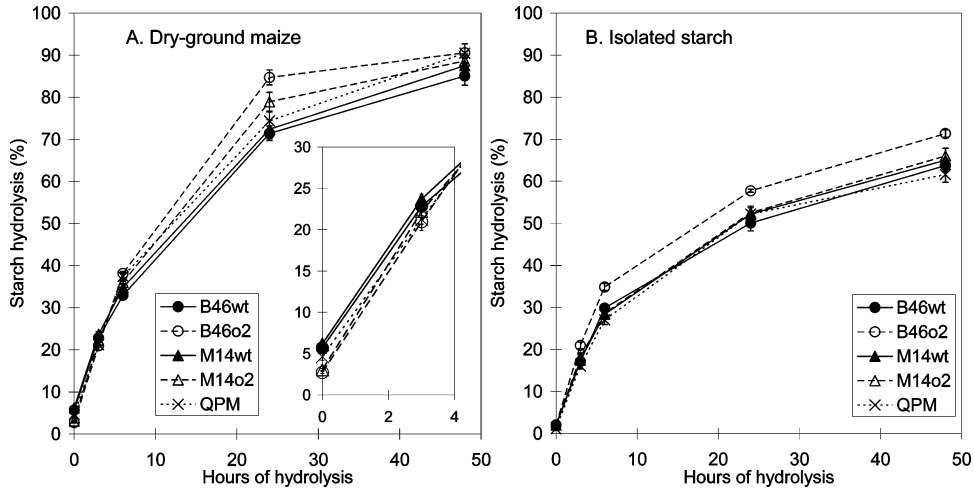

Figure 3. (**A**) Starch digestibilities of dry-ground WT, *o2*, and QPM maize and (**B**) that of isolated starches from whole kernels by wet milling. The inset in **A** shows a magnified plot of the first 4 h of starch digestibilities of the dry-ground maize samples. PPA was used for the digestibility studies.

Table 2. Amylose Contents of Isolated WT, *o2*, and QPM Starches

lines	amylose content (%)	
	iodine potentiometric titration	GPC
B46wt	33.7 ± 0.8	32.5 ± 1.1
B46o2	28.0 ± 2.7	28.9 ± 0.1
M14wt	32.9 ± 1.8	31.8 ± 0.9
M14o2	31.9 ± 0.2	31.0 ± 1.0
QPM	26.0 ± 2.0	28.5 ± 0.8

deionized water (5.0 mL) and equilibrated in a water bath at 37 °C with agitation for 10 min. PPA (125 units) in a phosphate buffer solution (0.10 M, pH 6.9, 5.0 mL) containing calcium chloride (0.25 mM) and sodium azide (0.04% w/v) was added to the starch or the dry-ground maize suspension. The mixture was vortexed and incubated in the same water bath for 48 h. Aliquots (0.7 mL) were removed from the hydrolysate after 0, 3, 6, 24, and 48 h of incubation. The reaction was stopped by adjusting the pH to 4.0 using a HCl solution (1.0 M, 20 μL) and was then adjusted to pH 6.0 using a NaOH solution (1.0 M). The aliquots were centrifuged at 6600*g* for 15 min. The supernatants (0.10 mL) containing soluble dextrin were further digested at 50 °C for 1 h with GA (0.25 units) in an acetate buffer solution (200 mM, pH 4.5, 0.90 mL) containing sodium azide (0.02% w/v). The glucose content of the supernatant after GA hydrolysis was quantified using a D-Glucose assay (GOPOD) kit. The starch digestibility was determined in triplicate. The enzyme digestibility was calculated as:

$$\text{starch digestibility} = \frac{\text{amount of glucose produced}}{\text{initial dry weight of starch}} \times 0.9$$

Amylose Content of Starch. *Iodine Potentiometric Titration.* The amylose content of starch was determined on the basis of the iodine affinity of defatted starch. The analysis was done in triplicate using an automatic potentiometric titrator (702 SM Tirino, Metrohm, Herisau, Switzerland) (*16*). Starch was defatted using an aqueous methanol solution (85% v/v) in a Soxhlet extractor for 24 h.

Gel Permeation Chromatography (GPC). The molecular weight distribution of starch molecules was analyzed using GPC (*16*), and the amylose content was calculated on the basis of molecular weight, that is, the second peak in the GPC profile. Starch (15 mg) was wetted with water (0.15 mL) and then mixed with dimethyl sulfoxide (1.35 mL). The starch dispersion was heated in a boiling water bath with stirring for 1 h and then stirred at room temperature for an additional 16 h. Starch was precipitated using 4 volumes of absolute ethanol, centrifuged, and redispersed in water (5 mL) in a boiling water bath with stirring for 30 min. The starch dispersion was filtered through a nylon membrane of 5.0 μm pore size and then injected into a GPC column (2.6 cm i.d. × 80 cm) packed with Sepharose CL-2B gel (Pharmacia, Inc., Piscataway, NJ). The column was eluted with an aqueous solution containing 25 mM sodium chloride and 1 mM sodium hydroxide in an ascending direction. Fractions (4.8 mL) of the eluent were collected. The total carbohydrate content (CHO) was determined using phenol and sulfuric acid (*17*). The blue value (BV) was obtained using iodine/potassium iodide solution (*18*). The colors developed from CHO and BV analyses of each fraction were quantified using a microplate reader (EL_X808, Bio-Tek Instruments, Inc., Winooski, VT) at 490 and 630 nm, respectively. The analysis was done in duplicate.

Solubility and Swelling Power. The solubility and the swelling power were analyzed by heating an aqueous suspension of starch (1% w/v, db, 5.0 mL) or dry-ground maize containing the same amount of starch in a shaker water bath at 80 °C and 120 rpm for 40 min (*19*). The analysis was done in duplicate.

Branch-Chain Length Distribution of Amylopectin. Amylopectin was fractionated from amylose using *n*-butanol and was then debranched using isoamylase (*20*). The debranched chains were labeled with 8-amino-1,3,6-pyrenetrisulphonic acid (APTS), and the branch-chain length distribution was analyzed using capillary electrophoresis (P/ACE MDQ, Beckman Courter, Fullerton, CA) (*21*). Maltohexaose and maltoheptaose were used as reference standards. The analysis was done in duplicate.

Starch Crystallinity. The X-ray diffraction pattern of the starch and the percentage of crystallinity were determined using a D-500 diffractometer (Siemens, Madison, WI) (*22*). The diffractometer was operated at 27 mA and 50 kV. The scanning region of the two-θ angle (2θ) was from 4 to 40° at 0.05° step size with a count time of 2 s.

Thermal Properties of Starch. The thermal properties of native isolated starch and retrograded starch were analyzed using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) (*16*). The starch sample was heated from 10 to 110 °C at a rate of 10 °C/min. An empty pan was used as the reference pan, and indium was used as a reference standard. The gelatinized-starch sample was kept in a refrigerator at 4 °C for 7 days and reanalyzed to characterize the retrograded starch. The analysis was done in triplicate.

$$\text{retrogradation (\%)} = \frac{\Delta H \text{ of the melting of retrograded starch}}{\Delta H \text{ of starch gelatinization}} \times 100\%$$

Statistical Analysis. Mean values of the kernel compositions were analyzed using analysis of variance (ANOVA) with the General Linear Model procedure in SAS version 9.1 (SAS Institute, Inc., Cary, NC). Differences were evaluated by *t*-test using Tukey's adjustment. The significance level was set at p value <0.05.

RESULTS

Kernel Composition. Starch, protein, and lipid contents of kernels of the different maize lines are shown in **Table 1**. Both *o2* mutants contained less protein than their WT counterparts.

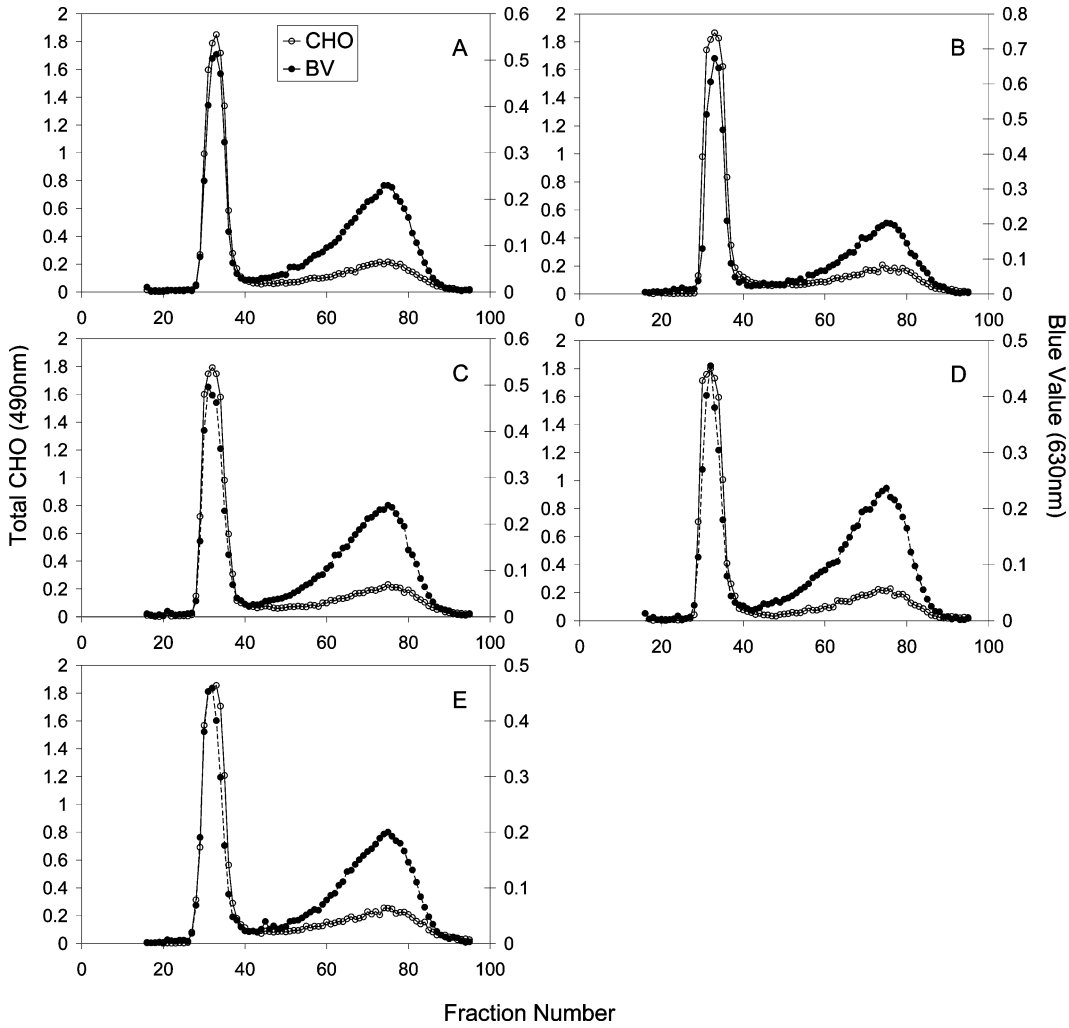

Figure 4. Molecular weight distribution of isolated (**A**) B46wt, (**B**) B46*o2*, (**C**) M14wt, (**D**) M14*o2*, and (**E**) QPM starches using Sepharose-CL-2B gel permeation chromatography. CHO (○) and BV (●). The first peak is designated to amylopectin, and the second peak is designated to amylose.

Table 3. Solubility and Swelling Power of Dry-Ground WT, *o2*, and QPM Maize and the Isolated Starches from Whole Kernels by Wet Milling[a]

	dry-ground maize		wet-milled starch	
lines	solubility (%)	swelling power (g/g)	solubility (%)	swelling power (g/g)
B46wt	10.3 ± 0.7 b	13.4 ± 0.5 ab	22.0 ± 1.5 b	15.4 ± 0.1 bc
B46o2	12.7 ± 0.2 c	16.0 ± 0.7 b	16.7 ± 0.5 a	14.2 ± 0.2 b
M14wt	9.6 ± 0.5 b	13.7 ± 1.0 ab	20.6 ± 0.2 ab	16.2 ± 0.2 c
M14o2	14.2 ± 0.5 c	16.7 ± 0.1 b	20.2 ± 1.0 ab	15.5 ± 0.5 c
QPM	5.2 ± 0.2 a	12.9 ± 0.7 a	17.1 ± 1.6 a	13.0 ± 0.4 a

[a] Values with the same letter in the same column are not significantly different at $p < 0.05$.

The severe *o2* mutant B46*o2* contained less starch than B46wt. The apparent larger lipid content of the B46*o2* maize kernels could be a result of the decreases in both protein and starch contents of the kernels.

Starch Granule Morphology. SEM images of starch granules isolated from dry-ground maize and isolated from whole kernels (isolated starch) by wet milling are shown in **Figures 1** and **2**, respectively. The starch granules isolated from the dry-ground WT maize and QPM (**Figure 1A**,**C**,**E**) showed more mechanical damage (debris and broken granules) than those isolated from the dry-ground *o2* maize (**Figure 1B**,**D**). Hila of some starch granules isolated from the dry-ground WT maize and QPM were exposed to the surface. The exposed hila were indicated with arrows (**Figure 1A**,**C**−**E**).

Starch granules isolated by wet milling of steeped whole kernels (**Figure 2**), however, did not show mechanical damage as observed with those isolated from the dry-ground maize (**Figure 1**). The B46wt starch showed more polygonal-shaped granules (**Figure 2A**) than the B46*o2* starch (**Figure 2B**), and the B46*o2* starch had more spherical granules. In contrast, the M14WT and the M14*o2* starches showed similar starch granule morphology (**Figure 2C**,**D**, respectively), which was attributed to the mild mutation of M14*o2*. The QPM starch had some irregular-shaped granules, which were larger than starch granules of other maize lines (**Figure 2E**). Some WT starch granules showed dimplelike indentations (marked with asterisks), which were not observed in the *o2* and the QPM starch granules. The B46*o2* and the QPM starch granules showed significantly more pinholes (marked with arrows) than the other maize starch granules.

Starch Digestibility. Digestibility of starch in the dry-ground maize and the isolated starch using PPA are shown in **Figure 3A**,**B**, respectively. In the first 3 h of the PPA hydrolysis, the rates of starch digestibility of the dry-ground WT-maize samples were slightly higher than those of the dry-ground *o2*-maize and QPM samples (**Figure 3A**, inset). After 6 h of hydrolysis, the dry-ground *o2*-maize and QPM samples displayed substantially greater starch hydrolysis than did the dry-ground WT-maize samples (**Figure 3A**).

For enzyme hydrolysis of isolated-starch samples, the severe *o2* mutant B46*o2* starch showed the greatest rate of hydrolysis

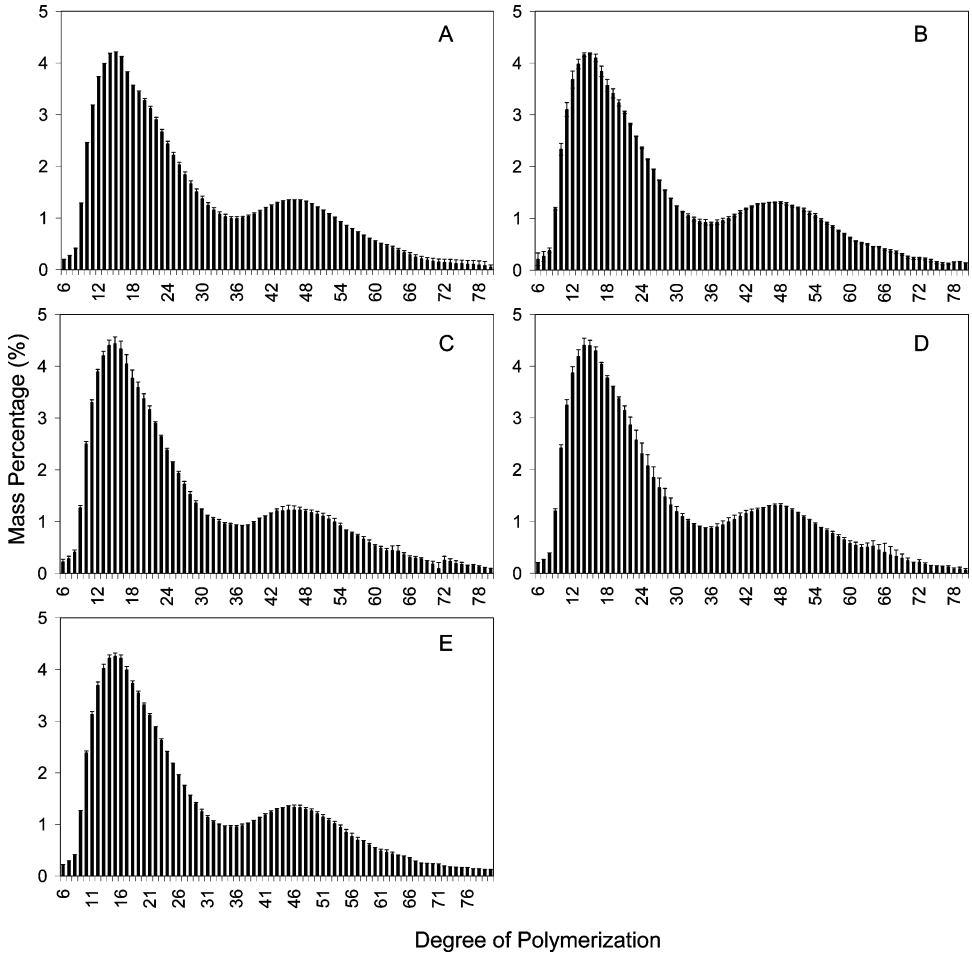

Figure 5. Branch-chain length distributions of amylopectins of (**A**) B46wt, (**B**) B46*o2*, (**C**) M14wt, (**D**) M14*o2*, and (**E**) QPM starches using fluorophore-assisted capillary electrophoresis.

Table 4. Branch-Chain Length Distribution of Amylopectins of WT, *o2*, and QPM Starches Analyzed Using Fluorophore-Assisted Capillary Electrophoresis

	branch-chain length distribution				
lines	DP 6−12 (%)	DP 13−24 (%)	DP 25−36 (%)	DP > 36 (%)	average DP
B46wt	11.5 ± 0.0	41.7 ± 0.3	17.1 ± 0.6	29.7 ± 0.8	21.2 ± 0.1
B46o2	11.2 ± 0.1	41.3 ± 0.7	15.9 ± 0.5	31.6 ± 1.1	21.5 ± 0.2
M14wt	11.9 ± 0.3	43.2 ± 1.2	15.9 ± 0.1	29.0 ± 1.4	20.9 ± 0.3
M14o2	11.6 ± 0.3	43.0 ± 0.1	15.2 ± 1.2	30.2 ± 0.9	21.1 ± 0.1
QPM	11.4 ± 0.2	42.3 ± 0.6	16.1 ± 0.0	30.2 ± 0.7	21.2 ± 0.2

(**Figure 3B**). Other isolated-starch samples showed similar digestibility rates. At the end of 48 h PPA hydrolysis, the mild *o2* mutant M14*o2* starch showed the second largest hydrolysis. All dry-ground maize samples displayed greater starch digestibility than the isolated-starch samples.

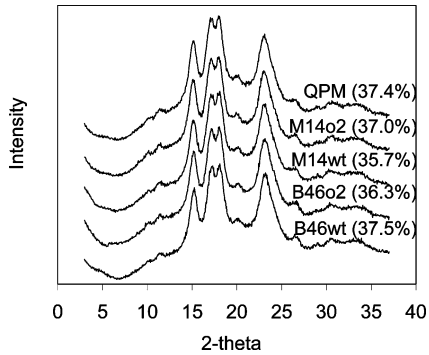

Figure 6. X-ray diffraction of isolated WT, *o2*, and QPM starches. Numbers in parentheses are the percentage of crystallinity.

Amylose Content of Starch. Amylose contents of the isolated-starch samples determined using iodine potentiometric titration and using GPC are shown in **Table 2**. GPC profiles of the isolated-starch samples are shown in **Figure 4**. The first peak of the GPC profile was amylopectin, and the second peak was amylose, which developed a darker blue color when stained with iodine. The amylose contents determined using GPC were similar to those determined using iodine potentiometric titration. The B46*o2* and the QPM starches had the lowest amylose contents.

Solubility and Swelling Power. The solubility and the swelling power of the dry-ground and the isolated-starch samples are shown in **Table 3**. The dry-ground *o2* had greater solubility and swelling power than the dry-ground WT-maize and QPM samples. For isolated-starch samples, the B46*o2* and the QPM starches had the lowest solubility and swelling power.

Branch-Chain Length of Amylopectin. The branch-chain length distributions of debranched amylopectin are shown in **Figure 5** and are summarized in **Table 4**. The percentages of branch chains of DP 6−12 were similar for all five amylopectin samples. The B46wt and B46*o2* amylopectin exhibited the least percentages of branch chains of DP 13−24, whereas the M14wt and M14*o2* amylopectin exhibited the largest. The average DPs of amylopectins of all maize lines were around 21.

Table 5. Thermal Properties of Isolated WT, *o2*, and QPM Starches[a]

	starch gelatinization				melting of retrograded starch				
lines	T_o[b] (°C)	T_p (°C)	T_c (°C)	H (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	H (J/g)	retrogradation (%)
B46wt	65.9 ± 0.1	70.3 ± 0.1	75.0 ± 0.1	13.1 ± 0.6	40.8 ± 0.4	50.8 ± 0.7	60.6 ± 0.1	6.6 ± 0.2	50.1 ± 1.1
B46o2	65.7 ± 0.3	71.8 ± 0.4	77.5 ± 0.4	14.9 ± 0.6	41.9 ± 0.3	51.9 ± 0.2	61.6 ± 0.5	7.2 ± 0.1	48.5 ± 1.2
M14wt	65.9 ± 0.2	72.6 ± 0.4	78.5 ± 0.5	13.9 ± 0.3	43.3 ± 0.3	53.1 ± 0.5	61.9 ± 0.2	7.3 ± 0.2	52.6 ± 0.7
M14o2	67.4 ± 0.4	72.3 ± 0.6	78.6 ± 0.7	14.4 ± 0.8	43.5 ± 0.1	53.5 ± 0.3	61.4 ± 0.5	7.2 ± 0.1	49.9 ± 3.3
QPM	66.0 ± 0.1	70.8 ± 0.1	76.1 ± 0.1	13.8 ± 0.6	43.0 ± 0.5	52.7 ± 0.7	60.9 ± 0.7	6.9 ± 0.2	49.7 ± 2.6

[a] T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, and H = enthalpy change.

Starch Crystallinity. All starches showed the A-type X-ray diffraction pattern (**Figure 6**). The degrees of crystallinity were similar for all of the isolated-starch samples.

Thermal Properties of Starch. Thermal properties of the isolated-starch samples are shown in **Table 5**. The B46wt and B46*o2* starches had slightly lower gelatinization temperatures and melting temperatures of retrograded starch than the M14wt and M14*o2* starches.

DISCUSSION

Kernels of B46*o2* and M14*o2* contained less protein than their WT counterparts (**Table 1**), which could be attributed to the suppression of zein biosynthesis in the *o2*-maize mutants. The solubility and the swelling power of the dry-ground *o2*-maize samples were greater than those of the WT counterparts (**Table 3**), whereas the solubility and swelling power of the isolated *o2* starches were smaller than those of their respective WT counterparts (**Table 3**). On the basis of these results, the greater solubility and swelling power of the dry-ground *o2* maize were attributed to the larger albumin and globulin contents of the *o2* mutants. Albumin and globulin are more water soluble and swell more than zein (*23*).

The surface of some WT starch granules had dimplelike indentations (**Figure 2A**,**C**), which were results of spherical-shaped protein bodies present between closely packed starch granules during kernel development (*24*). After the protein bodies were removed, the indentations remained on the surface of starch granules. The smooth surface of the *o2* and QPM starch granules, without the dimplelike indentations (**Figure 2B**,**D**), suggested that either starch granules were not tightly packed in the kernel or protein bodies were absent in *o2*-maize kernels. The large concentrations of zein present in the protein matrices of the WT and QPM kernels functioned like cement and bound starch granules together tightly (*25*). During dry grinding, the protein matrices were very hard to break apart; thus, the starch granules yielded to the force of grinding and were broken, some with hila exposed (**Figure 1A**,**C**,**E**). This was similar to the broken starch granules observed in the dry-milled flour of hard wheat (*25*). The dry-ground *o2*-maize samples, however, contained fewer mechanically damaged starch granules (**Figure 1B**,**D**) than the dry-ground WT-maize and QPM samples. These results suggested that the weak protein matrices in the *o2* kernels were broken apart easily during dry grinding and resulted in less damage to starch granules.

The substantial reduction in the starch content of B46*o2*-maize kernels (**Table 1**) might result from early termination of the starch biosynthesis in the *o2* mutant (*8*). The spherical granules of the isolated B46*o2* starch (**Figure 2B**) resembled starch isolated from premature kernels during maize kernel development (*26*). The WT starches displayed more polygonal-shaped granules (**Figure 2A**,**C**), which were the results of starch granule deformation when they were vigorously growing and competing for limited space in the endosperm. The B46*o2* starch contained less amylose than did the B46wt starch (**Table 2**), which also resembled premature maize starch (*26*). It is known that amylose is more actively synthesized at the later stage of starch biosynthesis (*26*−*28*), and the amylose content of starch is negatively correlated with starch susceptibility to amylase hydrolysis (*27, 29*). The large number of pinholes on the surface of the B46*o2* starch granules (**Figure 2B**), resulting from endogenous amylase hydrolysis (*26, 29*), and the greater starch digestibility of the B46*o2* maize (**Figure 3A**,**B**) could be attributed to its lower amylose content. The large numbers of pinholes on the surface of the B46*o2* and QPM starch granules also resulted in low swelling power and solubility of the two starches (*30*) (**Table 3**).

The B46wt and B46*o2* starches displayed slightly lower gelatinization temperatures than the M14wt and M14*o2* starches (**Table 5**), which correlated to fewer branch chains of DP 13−24 in the amylopectins of B46wt and B46*o2* starches (**Table 4**). It has been proposed that a decrease in branch chains of DP 13−24 causes defects in the amylopectin crystalline structure and results in a lower gelatinization temperature (*31*). The defects in the crystalline structure of starch granules also increased the susceptibility of the B46*o2* starch to PPA hydrolysis (**Figure 3B**).

The QPM starch granules contained the lowest amylose content (**Table 2**) and showed the largest number of pinholes on the granule surface (**Figure 2E**). The isolated QPM starch, however, displayed less PPA hydrolysis than the isolated B46*o2* starch. This could be related to its large starch-granule size, which had smaller relative surface space for enzymes to hydrolyze (*32*). It is known that starch granules with less amylose contents and smaller granular sizes are more quickly digested (*29*).

The fact that the dry-ground WT-maize samples were more susceptible to PPA hydrolysis during the first 3 h of digestion (**Figure 3A**, inset) could be a consequence of the greater proportions of mechanically damaged starch granules in those samples (*32*). After 6 h of PPA hydrolysis, the starches of the dry-ground *o2* maize, however, were hydrolyzed to greater extents than those of the WT counterparts. These observations were likely the results of highly digestible B46*o2* starch (**Figure 3B**) and the lower protein contents of the *o2* kernels (**Table 1**). Protein, surrounding starch granules, acts as a barrier for enzyme hydrolysis. The starch digestibility of the dry-ground QPM sample reached a similar level as that of the dry-ground B46*o2* sample after 48 h of PPA hydrolysis, which could relate to the low amylose content of the QPM starch (**Table 2**).

The percentage starch hydrolysis of the dry-ground maize (85−91%) was larger than that of the isolated starch (62−71%) after 48 h of enzyme hydrolysis using the same ratio of PPA to starch (**Figure 3A**,**B**, respectively). These could be attributed to the presence of mechanically damaged starch granules in the dry-ground maize samples (**Figure 1**), which were not observed in the isolated-starch samples (**Figure 2**). Furthermore, endogenous amylases present in the dry-ground maize, which were removed

from the isolated-starch samples, could also enhance the hydrolysis of the starch granules in the dry-ground maize (*33*).

In conclusion, mechanically damaged starch granules in the dry-ground WT maize were more susceptible to PPA hydrolysis at the first 3 h of digestion. After 6 h of digestion, starches in the dry-ground *o2*-maize and QPM samples were hydrolyzed faster than those in the dry-ground WT-maize samples because of reduced protein contents of the *o2* mutants and more digestible B46*o2* and QPM starches. The dry-ground maize also displayed larger starch digestibility than did the isolated starch, which was attributed to the presence of mechanically damaged starch granules and endogenous amylases in the dry-ground maize samples. The *o2* and QPM starches in dry-ground maize and as isolated starch were more readily digested by enzymes to provide energy for animal growth and to produce glucose for alcohol fermentation. Thus, the *o2* maize and QPM showed potential uses for feed and ethanol production.

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