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Physicochemical properties of endosperm and pericarp starches during maize development

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Abstract

Endosperm starch and pericarp starch were isolated from maize (B73) kernels at different developmental stages. Starch granules, with small size (2–4 μm diameter), were first observed in the endosperm on 5 days after pollination (DAP). The size of endosperm-starch granules remained similar until 12DAP, but the number increased extensively. A substantial increase in granule size was observed from 14DAP (diameter 4–7 μm) to 30DAP (diameter10–23 μm). The size of starch granules on 30DAP is similar to that of the mature and dried endosperm-starch granules harvested on 45DAP. The starch content of the endosperm was little before 12DAP (less than 2%) and increased rapidly from 10.7% on 14DAP to 88.9% on 30DAP. The amylose content of the endosperm starch increased from 9.2% on 14DAP to 24.2% on 30DAP and 24.4% on 45DAP (mature and dried). The average amylopectin branch chain-length of the endosperm amylopectin increased from DP23.6 on 10DAP to DP26.9 on14DAP and then decreased to DP25.4 on 30DAP and DP24.9 on 45DAP. The onset gelatinization temperature of the endosperm starch increased from 61.3 °C on 8DAP to 69.0 °C on 14DAP and then decreased to 62.8 °C on 45DAP. The results indicated that the structure of endosperm starch was not synthesized consistently through the maturation of kernel. The pericarp starch, however, showed similar granule size, starch content, amylose content, amylopectin structure and thermal properties at different developmental stages of the kernel.

Keywords: Corn starch; Endosperm starch; Pericarp starch; Starch development

1. Introduction

Maize is one of the most important crops extensively cultivated in the US. Mature maize kernels consist of up to 78% starch (Watson, 2003). Mature endosperm starches of maize and other cereal crops have been well studied for their compositions, structures, and properties. Starch structures and properties during kernel development, however, are not fully understood. Although the activities of many starch biosynthetic enzymes have been analyzed within the developing kernels of cereals, there is no integrated understanding of the relative roles of the expression of the starch biosynthetic enzymes to starch structures at different developmental stages.

Starch biosynthesis requires a series of enzymes working coordinately, including ADP-glucose pyrophosphorylase, granule-bound starch synthase (GBSS), soluble starch synthases, branching enzymes (BE) and debranching enzymes (DBE). Studies on starch biosynthesis have shown that each starch biosynthetic enzyme exists in several isoforms. The expression of certain isoform is tissue-specific. For example, ADP-glucose pyrophosphorylase mostly exists as an extra-plastidial form in the endosperm, whereas it is in a plastidial form in other tissues of cereal plants (James, Denyer, & Myers, 2003). In waxy wheat grains, GBSSI was knocked out from the endosperm tissue resulting in waxy wheat starch produced in the endosperm, but GBSS II was found in the pericarp, and thus normal starch was produced in the pericarp (Nakamura, Vrinten, Hayakawa, & Ikeda, 1998). Maize BEIIb was only found in the endosperm

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and other reproductive tissues (James et al., 2003). Even in the same tissue, the expression patterns of different enzyme isoforms are different. For example, BEIIa is expressed at the maximal level (5–7 days after flowering, DAF) earlier than BEI and BEIIb (7-10 DAF) in rice endosperm (Mizuno et al., 2001). Rice starch synthase (SS) III-1, SSIII-2, and SSIV-1 are expressed to the maximum level at the early, mid, and late developmental stages of the endosperm, respectively (Dian, Jiang, & Wu, 2005). In addition, different enzyme isoforms function differently in the starch biosynthesis. For example, maize branching enzyme I (BEI) catalyzes the transfer of longer chains than BEII (Guan & Preiss, 1993).

Starch is synthesized in either storage or transit form. As mentioned above, the expression patterns of starch biosynthetic enzymes differ in the storage organ and in other tissues. It suggests that starches produced in different tissues and at different time have different structures. Differences in structures of starches produced in different tissues have been reported in waxy cereals. Starch in endosperm, embryo sac and pollen of waxy kernel is stained brown–red with iodine, whereas starch in leaves and pericarp is stained black–blue (Hixon & Brimhall, 1968). In the storage organ of maize and potato, the amylose content increases with the increase in size and the radial distance from the hilum (Jane & Shen, 1993; Pan & Jane, 2000). Chemical gelatinization of normal maize and normal potato starch granules reveal that the amylose content is greater at the periphery than at the core of the starch granule, and amylopectin had shorter long-branch-chains at the periphery than at the core (Jane & Shen, 1993; Pan & Jane, 2000). These results suggest that starch structures change during the development of granules. However, there is lack of understanding on detailed structures of starch at different developmental stages and in different tissues.

In this study, we investigated starch granule morphology, the amylose content, amylopectin branch chain-length distribution and thermal properties of starches of endosperm and pericarp isolated at different kernel developmental stages. This information will be useful for the understanding of how enzymes catalyze starch biosynthesis and granule growth during the development of maize kernel.

2. Materials and methods

2.1. Materials

All chemicals were reagent grade and were purchased from Sigma Chemical Co. (St. Louis, MO). Crystalline *Pseudomonas* isoamylase (EC 3.2.1.68), specific activity about 66,000 units per milligram of protein, was purchased from Hayashibara Shoji, Inc. (Okayama, Japan) and was used without further purification.

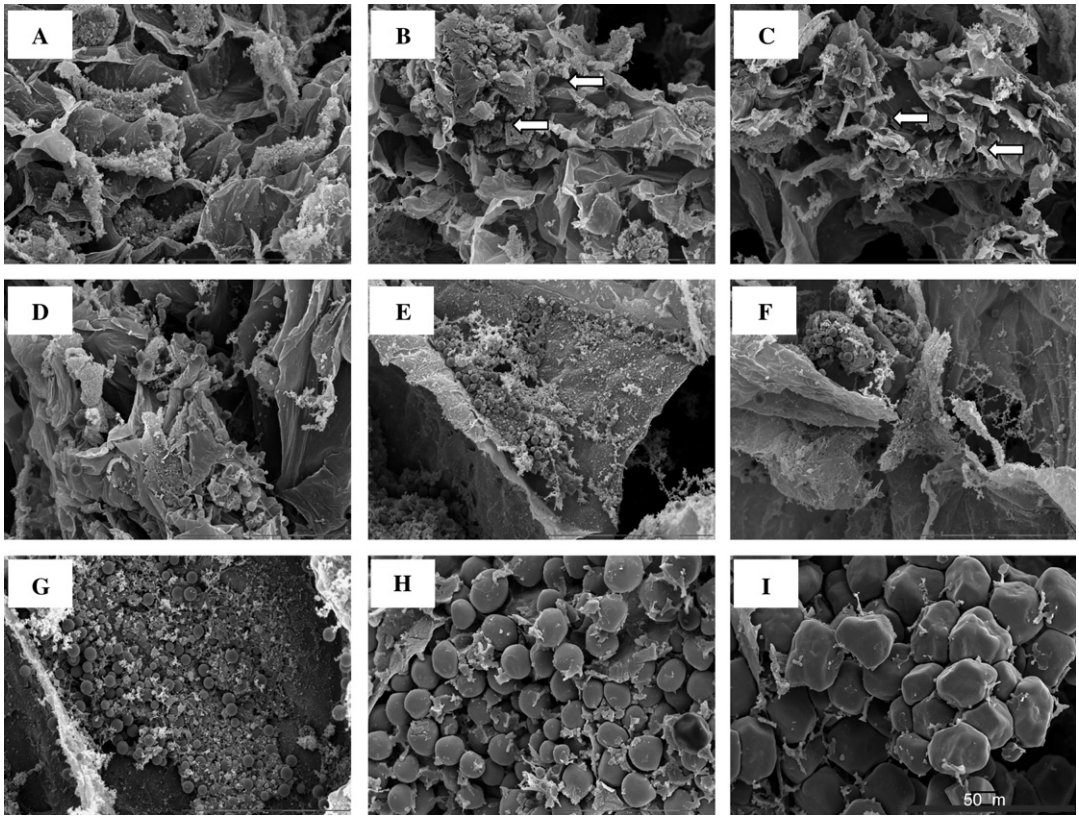

Fig. 1. Starch granules at the center of endosperms in the B73 maize kernels harvested at the different developmental stages (1500×). (A) Without pollination. (B) 5DAP, few starch granules are observed and are marked with arrows. (C) 6DAP. (D) 8DAP. (E) 10DAP. (F) 12DAP. (G) 14DAP. (H) 20DAP. (I) 30DAP.

2.2. Starch isolation

Maize kernels of self pollinated inbred B73 were harvested on 0, 5, 6, 8, 10, 12, 14, 20, and 30 days after pollination (DAP) and kept at −20 °C until starch extraction. Mature B73 maize starch was isolated from kernels harvested on 45DAP and dried at 35 °C for 5 days. The pericarp was separated from the endosperm by hand. Separated endosperms and pericarps were soaked in absolute ethanol to inactivate the enzymes. The steeped sample was milled in a microblender 3 × 1 min. The ground sample was filtered through a nylon screen with a pore size of 53 μm and washed with excess absolute ethanol. The residue was ground again with additional absolute ethanol until no more starch was released. Starch was collected by centrifugation, resuspended in 0.1 M aqueous NaCl solution containing 10% toluene and stirred for 1 h using magnetic stirrer at a high speed to remove protein. This step was repeated until the toluene layer became clear and contained no protein. The purified starch was washed three times with water and twice with ethanol and dried at 30 °C for 48 h.

2.3. Starch content of the maize endosperm and the pericarp

The starch contents of the endosperm and the pericarp were determined by using the Total Starch Kit (Megazyme, Co. Wicklow, Ireland). The separated endosperm and pericarp samples were freeze-dried before analysis. These samples were ground and then washed with 80% ethanol to remove any glucose residues. The sample was then digested with heat stable α-amylase and amyloglucosidase, mixed with GOPOD reagent (Megazyme, Co. Wicklow, Ireland), and measured at 510 nm. The sample was analyzed in duplicate. The moisture content of the sample was determined by drying the sample in an oven at 110 °C for 3 h.

2.4. Scanning electron micrographs of starch granules

Isolated starch granules and cracked maize kernels were mounted on the surface of a brass disk using double-sided adhesive silver-tape, coated with gold/palladium (60/40) and viewed under a scanning electron microscope (JOEL model 1850, Tokyo, Japan) at Bessey Electron Microscopy facility, Iowa State University. Micrographs of each starch sample were taken at 1500× or 5000× magnification.

2.5. Amylose content of starch

The amylose content of starch was determined by using gel permeation chromatography (GPC), following the method of Song and Jane (2000). Starch (15 mg) was wetted with 0.2 ml water and dispersed in DMSO (1.8 ml) in a boiling water bath, precipitated with ethanol, and then redispersed in boiling distilled water (5 ml). This starch dispersion was injected into a Sepharose CL-2B gel permeation (Pharmacia, Piscataway, NJ) column (1 cm

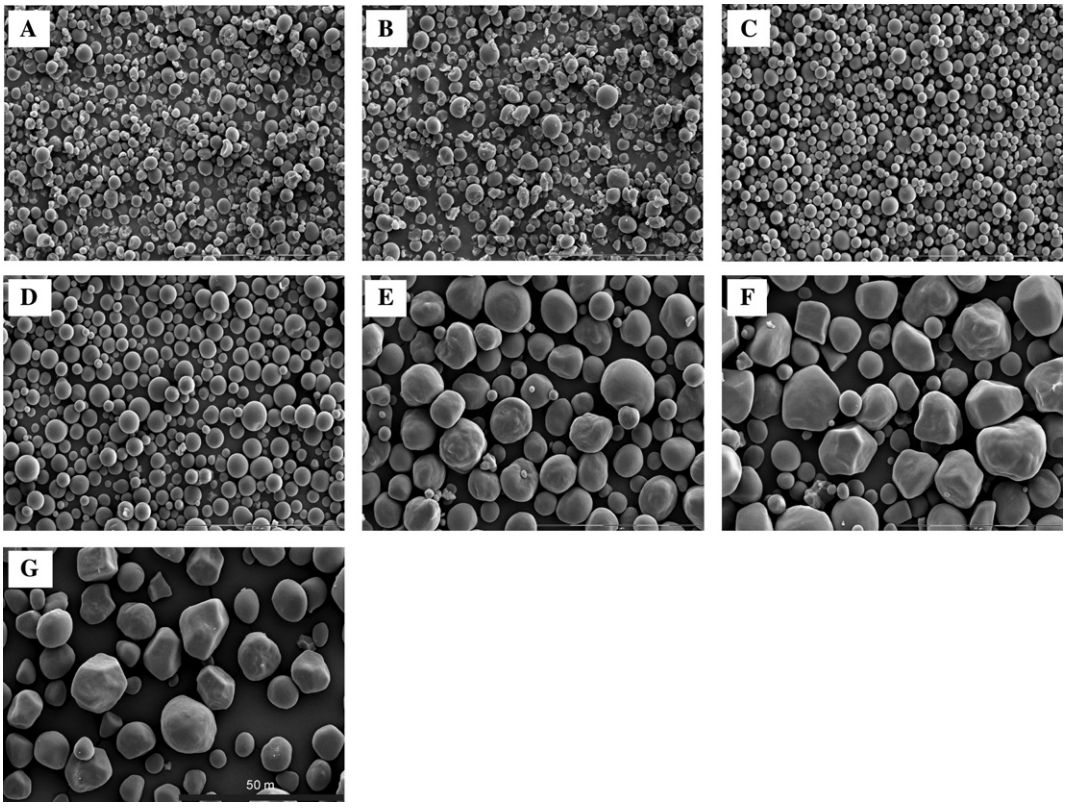

Fig. 2. SEM of endosperm starches isolated at different developmental stages (1500×). (A) 8DAP. (B) 10DAP. (C) 12DAP. (D) 14DAP. (E) 20DAP. (F) 30DAP. (G) 45DAP (mature and dried).

ID × 48 cm) and eluted by using an eluent containing 25 mM NaCl and 1 mM NaOH at a flow rate of 0.7 ml/min in a descending mode. Fractions of 1.0 ml each were collected and analyzed for total carbohydrate (Phenol-sulfuric acid method) (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and blue value (iodine staining) (Juliano, 1971) at 490 and 630 nm, respectively. The sample was analyzed in duplicate. The amylose content was calculated by dividing the total carbohydrate content of the amylose peak by the sum of that of the amylopectin and amylose peaks.

2.6. Amylopectin branch chain-length distribution

Amylopectin was separated from amylose using GPC. The fractions within the amylopectin peak were combined, evaporated, and used for branch chain-length distribution analysis. The amylopectin was dispersed in a DMSO solution (90%) and precipitated by adding 3 volumes of absolute ethanol and centrifuged at 8000*g* for 20 min. The amylopectin (3 mg) was re-dispersed in hot water (2.7 ml) and stirred in a boiling water bath for 20 min, cooled down to room temperature and digested with 60–120 units isoamylase in an acetate buffer solution (0.01 N, pH3.5) containing 0.02% sodium azide in a shaker water bath at 40 °C and 120 strokes per minute for 24 h. The digested sample was adjusted to pH 7 by adding NaOH and boiled for 15 min to inactivate the isoamylase.

The branch chain-length distribution of amylopectin was analyzed using high performance anion-exchange chromatography (Dionex-300, Sunnyvale, CA) equipped with an on-line amyloglucosidase column and an ED50 pulse-amperometric detector (Dionex, Sunnyvale, CA) (HPAEC-ENZ-PAD). The debranched amylopectin was separated using a PA-100 anion-exchange analytical column (4 × 250 mm) and a guard column (Dionex, Sunnyvale, CA). The operating condition was the same as that described by McPherson and Jane (1999). The data were analyzed by using Chromeleon software (Dionex, Sunnyvale, CA), and the sample was analyzed in duplicate.

2.7. Thermal properties of starches

Thermal properties of isolated starches were determined using a differential scanning calorimeter (DSC-7, Perkin–Elmer, Norwalk, CT), following the method of Song and Jane (2000). The starch sample (about 3 mg, dry starch basis (dsb)) with excess water (1:3) was heated at 10 °C/min from 25 to 110 °C in sealed aluminum pans, using an empty pan as the reference. The sample was analyzed in triplicate, and the data were calculated using Pyris software (Perkin–Elmer, Norwalk, CT). The gelatinized starch sample was stored at 4 °C for 7 days and then analyzed following the same procedure to determine the properties of the retrograded starch and percentage retrogradation.

3. Results and discussion

3.1. Endosperm- and pericarp-starch granule developments in the kernel

Scanning electron micrographs (SEM) of cracked kernels are shown in Fig. 1. Fig. 1A shows the ovule before pollination. Few granules were first observed at the center of the endosperm on 5DAP, and the sizes of the starch granules were small (1–4 μm) (Fig. 1B). Starch granules of 1–4 μm diameters did not show Maltese cross under a polarized microscope, and they were stained blue with iodine solution (data not shown). Up to 12DAP (Figs. 1C–F), the size of starch granules remained similar and small, but the number of starch granules increased. A significant increase in starch granule size was observed on 14DAP (diameter about 7 μm) (Fig. 1G). It appeared that granules remained spherical on 20DAP with diameters about 10–16 μm (Fig. 1H). The granule sizes increased up to 23 μm and became polygonal shape on 30DAP (Fig. 1I). The polygonal shaped granules were results of space restriction. Starch granules located at the periphery of the endosperm had smaller sizes than that at the center

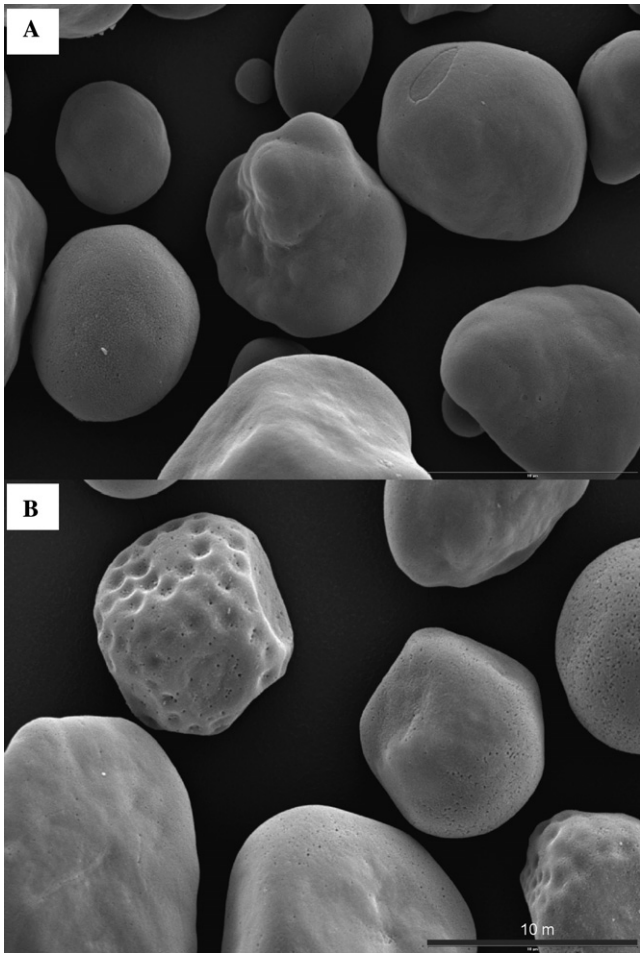

Fig. 3. SEM of endosperm starch isolated at 30DAP and mature endosperm starch (5000×). (A) 30DAP. (B) 45DAP (mature and dried).

and maintained spherical shape. The size of starch granules on 30DAP (up to 23 μm) is similar to that of the mature and dried endosperm-starch granules harvested on 45DAP. SEM of isolated endosperm starches are shown in Fig. 2. Comparing with starch granules isolated on or before 30DAP, there were substantially large numbers of pinholes observed on the surface of the mature and dried endosperm-starch granules harvested on 45DAP (Fig. 3). The pinholes could be attributed to endogenous enzyme hydrolysis during maturation in the field and drying at 35 °C.

The results of the increase in the number of starch granule up to 12 DAP and the increase in the size of starch granules from 14DAP to 30DAP in the maize endosperm were similar to those observed in rice endosperm by Briones, Magbanua, and Juliano (1968). Development of starch granules have been studied in many plants of other species. Most plants show increases in the

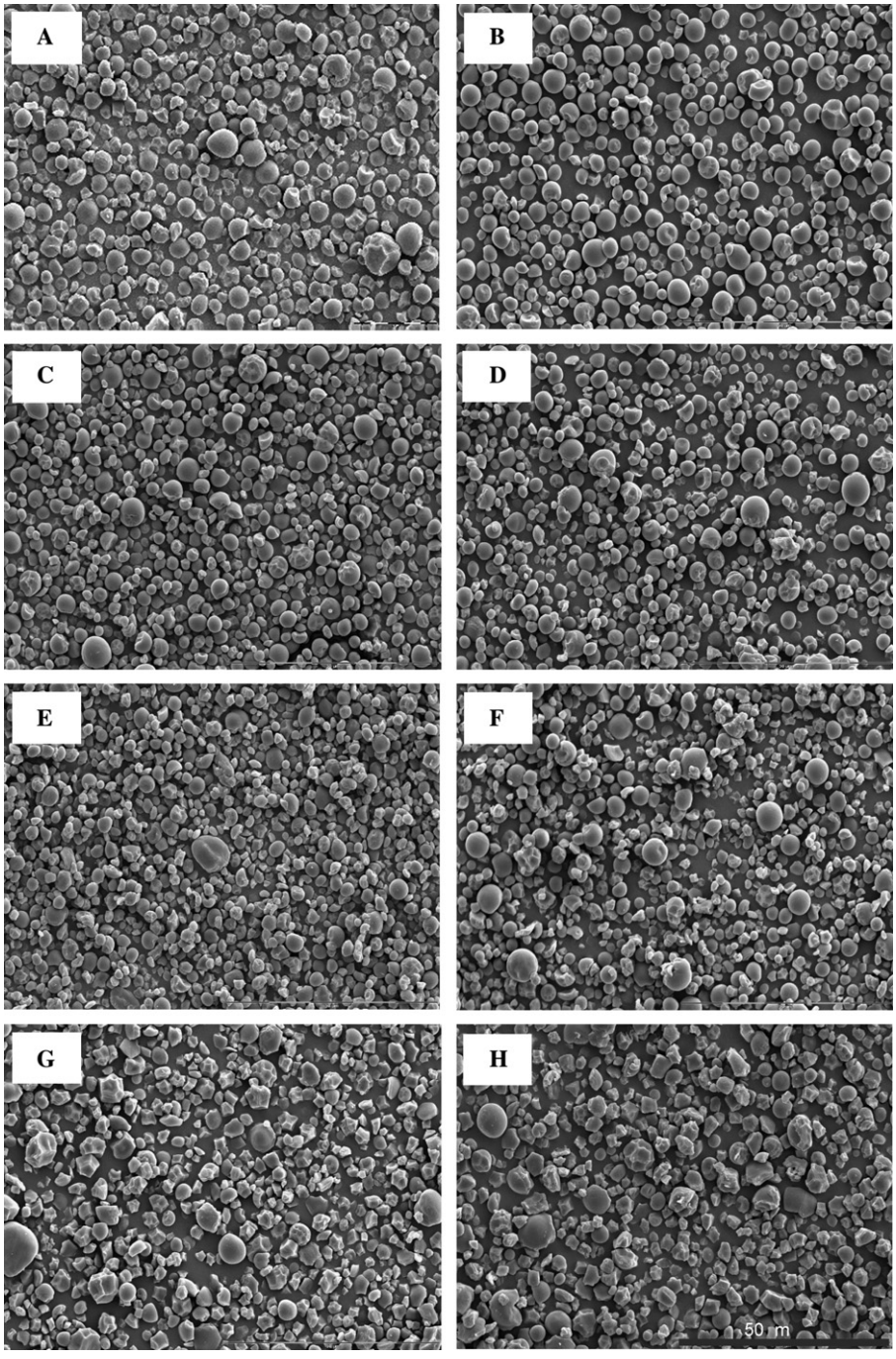

Fig. 4. SEM of pericarp starches isolated at different developmental stages (1500×). (A) Ovule (without pollination). (B) 6DAP. (C) 8DAP. (D) 10DAP. (E) 12DAP. (F) 14DAP. (G) 20DAP. (H) 30DAP.

size of starch granules during the maturation of storage organs, including endosperms of rice (Murugesan & Hizukuri, 1992) and barley (Duffus & Cochrane, 1993), and tubers of yam (Sugimoto, Nishihara, Abe, Fujita, & Fuwa, 1987), potato (Liu, Weber, & Yada, 2003), and arrowhead (Sugimoto, Yamamoto, Abe, & Fuwa, 1988). Starch granules of taro (Sugimoto, Yamamoto, Abe, & Fuwa, 1987), however, display similar sizes and shapes throughout the development of the root.

The biosynthesis of pericarp-starch granules, however, was independent form the kernel development and maintained similar sizes (2–4 μm) during kernel maturation. The shapes of pericarp-starch granules were spherical at the early stage of the kernel development, but became polygonal and irregular shapes at the later stage of the kernel development (Fig. 4). The shape change could be caused by the pressure built up from the growth in the size of the endosperm. The pericarp was evolved from the ovary wall after pollination. The size increase in the pericarp was accompanied by its cell division and enlargement during the kernel development (Watson, 2003). The pericarp starch might be used as an energy source for the growth of the pericarp, which was not controlled by pollination.

3.2. Starch contents of the endosperm and pericarp

The starch content of the maize endosperm and pericarp are shown in Table 1. The starch content of maize endosperm increased from 1.0% on 8DAP, 2.0% on 12DAP, 10.7% on 14DAP, and 88.9% on 30DAP. These results were in agreement with the activities of starch biosynthetic enzymes in the developing maize endosperm (Tsai, Salamini, & Nelson, 1970). The enzyme activities of UDP-glucose pyrophosphorylase, ADP-glucose pyrophosphorylase, hexokinase, granule bound starch synthase and soluble starch synthase all increased rapidly after 12DAP and reached the maximum on 20-22DAP. These studies indicated that the fast increase in the starch content of the endosperm after 12DAP was a result of the rapid increase in starch biosynthetic enzyme activities. In contrast, there was no significant difference in the starch content of pericarp during the kernel development except the lower starch content of unpollinated ovule (Table 1).

3.3. Amylose contents of the endosperm and pericarp starches

The amylose contents of the endosperm and pericarp starches are shown in Table 2. The amylose content of the endosperm starch increased from 9.2% on 12DAP to 24.2% on 30DAP. The mature and dried (45DAP) endosperm starch had a similar amylose content (24.4%) as the starch isolated on 30DAP. The results agreed with that the small granules contained less amylose than the large granules. Kidney bean starch (Yoshida et al., 2003), potato starch (Jane & Shen, 1993; Liu et al., 2003), and maize starch (Pan & Jane, 2000) show the same trend for starch of different granule sizes. This result was consistent with the report that the outer layer of starch granules, separated by chemical surface gelatinization, contained greater amylose content than the inner part of the granules (Jane & Shen, 1993; Pan & Jane, 2000). It is known that the starch granule is synthesized by apposition, from the hilum towards the periphery (Yoshida, Fujii, Nikuni, & Maruo, 1958). Thus, the core of the starch granule corresponds to the small granule synthesized at the early stage of the starch granule development.

Granule bound starch synthase I (GBSSI) is the primary enzyme for amylose biosynthesis in the storage organ (Vrinten & Nakamura, 2000). In the storage organs of pea and potato, the level of GBSSI expression

Table 1
Starch contents of the endosperm and the pericarp at different developmental stages of maize

Days after pollination	Endosperm (% dry weight)	Pericarp (% dry weight)
0	ND	4.1 ± 0.0 (Ovule)
8	1.0 ± 0.1	11.1 ± 0.8
10	1.5 ± 0.8	9.9 ± 0.1
12	2.0 ± 0.5	10.8 ± 1.2
14	10.7 ± 1.7	8.2 ± 2.8
20	68.3 ± 4.9	11.3 ± 0.0
30	88.9 ± 5.1	10.9 ± 0.4

ND: not detectable.

Table 2
Amylose contents of endosperm and pericarp starches at different developmental stages of maize

Days after pollination	Endosperm (%)[a]	Pericarp (%)
0	nd[b]	19.6 ± 0.8
6	nd	19.7 ± 1.9
8	nd	19.0 ± 1.3
10	nd	14.7 ± 0.9
12	9.2 ± 0.8	14.4 ± 1.4
14	11.1 ± 0.6	16.2 ± 3.5
20	21.4 ± 0.9	18.3 ± 1.2
30	24.2 ± 0.8	19.3 ± 2.3
45 (mature and dried)	24.4 ± 0.7	nd

[a] Values given are means ± standard deviation obtained from two replicates.
[b] Not determined.

Table 3
Branch chain-length distributions of endosperm amylopectins

Samples	Percent distribution				Average CL
Days after pollination	DP ⩽ 12	DP13–24	DP25–36	DP ⩾ 37	
10	21.1 ± 1.6[a]	48.3 ± 0.2	14.2 ± 0.0	16.3 ± 1.8	23.6 ± 0.9
12	21.1 ± 0.1	42.2 ± 0.1	15.8 ± 0.4	20.9 ± 0.2	24.8 ± 0.1
14	18.3 ± 0.1	45.6 ± 0.2	13.7 ± 0.5	22.5 ± 0.7	26.9 ± 0.2
20	16.7 ± 0.0	46.2 ± 0.1	15.1 ± 0.3	22.1 ± 0.3	26.3 ± 0.1
30	17.4 ± 0.5	47.5 ± 0.9	14.5 ± 0.3	20.6 ± 1.6	25.4 ± 0.6
45 (mature and dried)	19.4 ± 0.0	46.3 ± 0.8	13.4 ± 0.3	20.8 ± 1.0	24.9 ± 0.5

[a] Values given are means ± standard deviation obtained from two replicates.

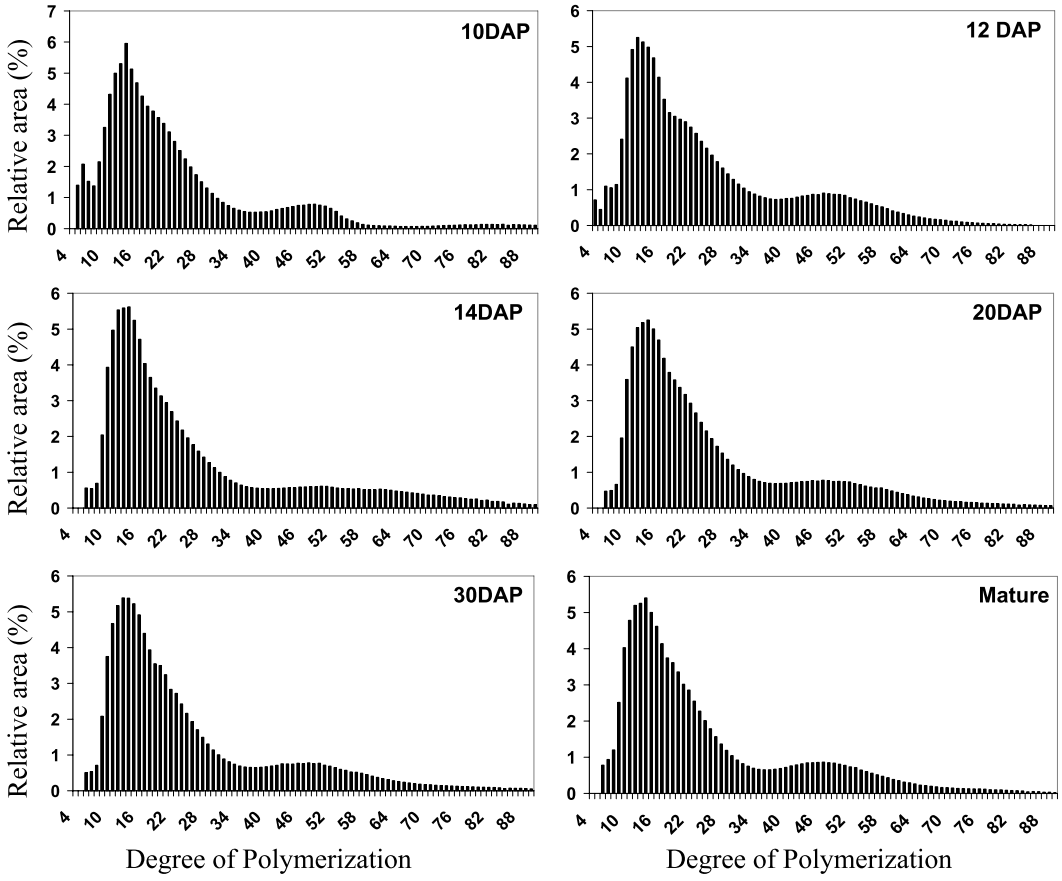

Fig. 5. Branch chain-length distributions of maize amylopectins isolated at different developmental stages.

increases in the later developmental stage (Dry et al., 1992). The mRNA of rice (Dian et al., 2005) and maize (Gao, Fisher, Kim, Shannon, & Guiltinan, 1996) GBSSI increased with the development of endosperm. Thus, the increase in GBSSI expression is directly related to the increase in the amylose content of starch during the maize kernel development.

Unlike that of the endosperm starch, the amylose content of pericarp starch did not increase with the kernel development but remained at a similar level and were lower on 10-14DAP. Using iodine staining, Hixon and Brimhall (1968) report that leaf and pericarp starches of the waxy mutant of maize stain black–blue, whereas endosperm and pollen starches of the same mutant stain brown–red. Similar results are also obtained in rice (Igaue, 1964) and wheat (Nakamura et al., 1998). Vrinten and Nakamura (2000) report that different isoforms of granule-bound starch synthases are present in the endosperm and the pericarp tissues of wheat, which explain the different amylose contents of the endosperm and pericarp starches (Table 2).

Table 4
Branch chain-length distributions of pericarp amylopectins

Samples	Percent distribution				Average CL
Days after pollination	DP ⩽ 12	DP13–24	DP25–36	DP ⩾ 37	
0	20.5 ± 0.7[a]	45.7 ± 1.1	13.8 ± 0.1	20.0 ± 1.9	25.1 ± 1.1
6	18.0 ± 0.7	44.2 ± 0.7	14.0 ± 0.3	23.8 ± 1.1	26.8 ± 0.5
8	19.6 ± 0.2	44.8 ± 0.4	13.6 ± 0.0	22.0 ± 0.6	25.9 ± 0.3
10	20.2 ± 0.5	45.9 ± 0.5	13.6 ± 0.3	20.4 ± 0.8	25.3 ± 0.4
12	21.3 ± 0.0	43.4 ± 0.4	11.1 ± 0.0	23.8 ± 0.5	28.3 ± 0.1
14	20.0 ± 0.2	46.5 ± 0.1	14.1 ± 0.1	19.2 ± 0.2	24.7 ± 0.1
20	21.0 ± 0.3	46.1 ± 1.1	13.9 ± 0.2	19.1 ± 1.3	24.5 ± 0.6
30	21.8 ± 0.1	47.0 ± 0.6	12.9 ± 0.0	18.2 ± 0.8	24.1 ± 0.3

[a] Values given are means ± standard deviation obtained from two replicates.

Table 5
Thermal properties of native endosperm starches

Samples[a]	Native starch			
Days after pollination	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
8[b]	61.3	66.5	73.2	13.0
10[b]	61.5	66.9	73.3	13.3
12	63.0 ± 0.2[c]	67.8 ± 0.3	74.8 ± 0.5	14.5 ± 0.2
14	69.0 ± 0.2	72.9 ± 0.2	77.8 ± 0.2	15.6 ± 0.5
20	67.5 ± 0.0	71.9 ± 0.0	77.7 ± 0.1	15.6 ± 0.3
30	67.4 ± 0.1	71.0 ± 0.2	75.3 ± 0.1	14.2 ± 0.3
45 (mature and dried)	62.8 ± 0.1	68.0 ± 0.1	72.8 ± 0.1	13.8 ± 0.1

[a] Samples (~3.0 mg, dsb) and deionized water (~9.0 mg) were used for the analysis; T_0, T_p, T_c and ΔH are onset, peak, conclusion temperature, and enthalpy change, respectively.
[b] Value was measured once.
[c] Values were calculated form three replicates; ±SD.

3.4. Amylopectin branch chain-length distributions

The branch chain-length distributions of endosperm starch amylopectins are shown in Table 3. The branch chain-length profiles showed a bimodal distribution with peak chain lengths at DP14 and DP47–50 (Fig. 5). The shortest branch chain detected was DP4 on 12DAP. The short chains (DP $\leqslant$ 12) of endosperm amylopectins decreased from 21.1% on 10DAP to 16.7% on 20DAP and then increased to 17.4% on 30DAP and 19.4% on 45DAP. Branch chains of DP13–24 decreased from 48.3% on 10DAP to 42.4% on 12DAP and then increased to about 46%f and 47% till mature. The long chains (DP $\geqslant$ 37) of endosperm amylopectins increased from 16.3% on 10DAP to 22.5% on 14DAP and then decreased to about 20.6% on 30DAP and mature. The same trend was also observed on the average chain length of endosperm amylopectins. Amylopectin of endosperm starch harvested on 14DAP showed the longest average chain length. These results indicated that the structures of amylopectin molecules were not homogenous through the starch granule but changed with the development of the granule and the endosperm.

BEI and BEIIb in maize W64A display different expression patterns during the kernel development (Gao et al., 1996). The ratio of the transcript levels of BEI to BEIIb is larger at the early kernel development, until 12DAP, and then decreased after 12DAP. Guan and Preiss (1993) have reported that maize BEI transfers longer branches than BEII *in vitro*. The increase in the relative level of BEIIb after 12DAP is likely to result in shorter average branch chain-length of amylopectin after 14DAP. At the later developmental stage (after 20DAP), the average branch-chain length and the percentage of long chains decreased. This is in agreement with that the long B chains of amylopectin at the periphery of potato and maize starch granule are shorter than those at the core of the granules reported by Jane and Shen (1993) and Pan and Jane (2000). The branch chain-length distributions of pericarp starch are shown in Table 4. There was no clear correlation between the branched structure of pericarp amylopectin and the kernel development.

3.5. Thermal properties

Thermal properties of endosperm starch measured by using DSC are shown in Table 5. The onset gelatinization temperature of endosperm starch increased from 61.3 °C on 8DAP and 61.5 °C on 10DAP to 69.0 °C on 14DAP and then decreased to 67.4 °C on 30DAP and 62.8 °C for mature and dried sample (45DAP). The low gelatinization temperature of starch produced on 10DAP agreed with its shortest average branch chain length of amylopectin, the smallest amount of the long chains (DP $\geqslant$ 37), and the largest proportion of short chains (DP $\leqslant$ 12). Studies have shown that

Table 7
Thermal properties of native pericarp starches

Samples[a]	Native starch			
Days after pollination	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
0	55.9 ± 0.8[b]	64.5 ± 0.5	72.9 ± 0.7	15.2 ± 0.2
6	63.5 ± 0.3	69.1 ± 0.3	75.3 ± 0.4	14.9 ± 0.6
8	61.2 ± 0.6	67.0 ± 0.8	74.2 ± 1.0	14.2 ± 0.4
10	61.6 ± 0.6	67.3 ± 0.5	74.4 ± 1.3	14.9 ± 0.9
12	60.8 ± 0.4	66.2 ± 0.5	71.0 ± 3.1	14.4 ± 0.4
14	60.8 ± 0.2	66.6 ± 0.4	75.2 ± 0.9	15.3 ± 0.7
20	59.4 ± 0.4	65.8 ± 0.5	72.7 ± 0.9	14.3 ± 0.3
30	62.6 ± 0.5	68.6 ± 0.5	75.9 ± 0.7	14.7 ± 0.7

[a] Samples (~3.0 mg, dsb) and deionized water (~9.0 mg) were used for the analysis; T_0, T_p, T_c and ΔH are onset, peak, conclusion temperature, and enthalpy change, respectively.
[b] Values were calculated form three replicates; ±Standard deviation.

Table 8
Thermal properties of retrograded pericarp starches

Samples[a]	Retrograded starch				Retrogradation (%)[b]
Days after pollination	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	
0	42.8 ± 1.0	53.1 ± 0.4	61.3 ± 0.1	4.3 ± 0.1	28.1 ± 0.2
6	42.1 ± 0.4	55.2 ± 1.3	63.0 ± 0.7	4.6 ± 0.7	30.9 ± 3.0
8	42.5 ± 0.5	55.6 ± 1.6	63.0 ± 0.9	4.4 ± 0.3	31.0 ± 3.1
10	43.0 ± 1.2	54.8 ± 0.4	63.7 ± 0.4	4.6 ± 0.4	31.1 ± 4.0
12	42.0 ± 1.3	55.4 ± 1.1	63.9 ± 0.7	4.0 ± 0.3	27.7 ± 3.1
14	42.9 ± 0.7	54.3 ± 0.2	63.3 ± 0.3	4.8 ± 0.7	31.6 ± 4.7
20	40.6 ± 0.2	54.3 ± 0.4	63.7 ± 0.4	3.9 ± 0.4	27.1 ± 3.2
30	40.4 ± 0.9	54.1 ± 0.3	62.7 ± 0.3	4.1 ± 0.4	27.8 ± 3.4

[a] After storage at 4 °C for 7 days.
[b] Retrogradation (%) = $\Delta H_{retro}/\Delta H_{native} \times 100$.

Table 6
Thermal properties of retrograded endosperm starches

Samples[a]	Retrograded starch				Retrogradation (%)[b]
Days after pollination	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	
8[c]	40.7	55.0	62.0	3.4	26.4
10[c]	41.0	54.7	61.2	3.3	24.8
12	41.1 ± 0.8	52.9 ± 0.8	62.8 ± 0.5	6.7 ± 0.3	46.1 ± 2.3
14	40.3 ± 0.6	52.0 ± 0.5	63.0 ± 0.5	7.6 ± 0.6	48.7 ± 3.3
20	40.7 ± 0.7	52.3 ± 0.5	63.9 ± 0.2	6.7 ± 0.2	42.9 ± 2.3
30	40.2 ± 0.9	52.0 ± 0.8	62.7 ± 0.8	6.1 ± 0.5	42.7 ± 4.4
45 (mature and dried)	36.3 ± 0.7	49.1 ± 0.3	61.0 ± 0.3	7.9 ± 0.3	57.4 ± 2.1

[a] After storage at 4 °C for 7 days.
[b] Retrogradation (%) = $\Delta H_{retro}/\Delta H_{native} \times 100$.
[c] Value was measured once. Other values were calculated form three replicates; ±SD.

starch having more short chains DP ⩽ 12 and less long B chains displayed lower gelatinization temperature (Jane, Shen, Chen, Lin, & Kasemsuwan, 1992, 1999; Shi & Seib, 1992; Yuan, Thompson, & Boyer, 1993). Vandeputte, Vermeylen, Geeroms, and Delcour (2003) have reported that the percentage of B1 chain (DP13–24) is positively correlated with the onset gelatinization temperature of rice starches, whereas that of the short chain (DP ⩽ 12) is negatively correlated with the onset gelatinization temperature of rice starches. The increase in the onset starch gelatinization temperature from 63.0 °C on 12DAP to 69.0 °C on 14DAP can be attributed to the decrease in short branch chains (DP ⩽ 12) from 21.1% to 18.3% and the increase in branch chains of DP13–24 from 42.2% to 45.6% (Table 3). The enthalpy change increased from 13.0 J/g on 8DAP to 15.6 J/g on 14 and 20DAP, indicating less crystallinity in the small granules than the large ones. This result was consistent with the observations that the hilum of a granule was loosely packed with less ordered structure (Baker, Miles, & Helbert, 2001; Pan & Jane, 2000). The onset gelatinization temperature of the endosperm starch decreased from 69.0 °C on 14DAP to 67.4 °C on 30DAP and 62.8 °C on 45DAP (mature and dried). The changes in the gelatinization temperature were related to the changes in the branch chain length.

The gelatinization temperature of the mature and dried starch (45DAP) was substantially lower than that of the starch samples harvested on earlier dates without drying. The large number pinholes observed on the surface of the mature and dried starch granules (Fig. 3) suggested that the starch granules were subjected to enzymatic hydrolysis. Alpha-amylase hydrolyzes amylose and amylopectin located in both amorphous and crystalline regions of the starch granule (Colonna, Buleon, & Lemarie, 1988). Further studies are needed to reveal if the enzyme attack affects the structure and property of the mature and dried starch.

The percentage retrogradation of endosperm starches increased from 26.4% on 8DAP to 48.7% on14DAP (Table 6). Increasing amylose content of the starch and increasing branch chain length of amylopectin were attributed to the increase in the percentage retrogradation. It is known that amylose molecules and long-branch chains of amylopectin retrograde quickly. The starch harvested on 45DAP showed a substantial increase in the percentage retrogradation, which could be attributed to the drying process of the kernels. The slow drying at 35 °C for 5 days could cause entanglement between starch molecules and enhanced retrogradation. The gelatinization temperature and the percentage retrogradation of pericarp starches did not change with the development of kernels except the low gelatinization temperature of the pericarp starch on 0DAP (Tables 7 and 8).

4. Conclusions

Maize endosperm starch content, starch granule size, and amylose content increased during the kernel development. Branch chain-length analysis of endosperm amylopectin showed shorter average chain length (DP23.6) on 10DAP, increased to the maximum (DP26.7) on 14DAP, and then decreased to DP25.4 on 30DAP and DP24.9 on 45DAP (mature and dried). The onset gelatinization temperature of endosperm starch increased from 61.3 °C on 8DAP to 69.0 °C on 14DAP and then decreased to 67.4 °C on 30DAP and 62.8 °C on 45DAP. In contrast, there were no significant changes in granule size, amylose content, starch content, and thermal properties of pericarp starches during the development of maize kernel.

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