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Carbohydrate Research 282 (1996) 157–170

CARBOHYDRATE RESEARCH

Maize starch fine structures affected by ear developmental temperature [1]

Ting-jang Lu [a], Jay-lin Jane [a,*], Peter L. Keeling [b,2], George W. Singletary [b,3]

[a] *Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011, USA*
[b] *ICI Seeds, Applied Biology Project, P.O. Box 500, Slater, IA 50244, USA*

Received 2 May 1995; accepted 24 October 1995

Abstract

Growing temperature is known to affect the grain yield and quality of maize. Two genetically unrelated normal dent maize inbreds, ICI63 and ICI92, with different heterotic backgrounds were grown in a greenhouse with the ears wrapped in temperature control devices set at 25 and 35 °C during the grain-filling period. Grain yield, kernel weight, and kernel density were less for ears at 35 °C than for those at 25 °C. The extent of the loss, however, varied with the variety: 13.1 and 37.9% kernel weight loss and 8.47 and 10.08% density loss for ICI63 and ICI92, respectively. The starch granular shape of ICI63 became more oval-shaped, but there was no shape change for ICI92. As developmental temperature increased, starch granule size decreased and gelatinization temperature increased. With increasing developmental temperature, the true amylose content of ICI63, determined by iodine affinity, decreased 2.39% and that for ICI92 decreased 2.20%; amylose molecular size of both varieties also decreased. Size exclusion chromatography and high-performance anion-exchange chromatography revealed an increased medium branch-chain fraction and decreased long and short branch-chain fractions for ICI63 amylopectin, whereas ICI92 amylopectin had increased long and medium branch-chain fractions and a decreased short branch-chain fraction, when the ear developed at 35 °C.

Keywords: Maize; Starch; Amylose; Amylopectin

* Corresponding author.

[1] Journal Paper No. J-16320 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, USA, Project No. 3258.

[2] Present address: ExSeed Genetics L.L.C., 1573 Food Science Building, Iowa State University, Ames, IA 50011, USA.

[3] Present address: Pioneer Hi-Bred International, Inc., P.O. Box 1004, Johnston, IA 50131, USA.

SSDI 0008-6215(95)00370-3

1. Introduction

Temperature is one environmental variable that cannot be manipulated in the field, and crops are often selected for a region on the basis of regional temperature. Temperature fluctuations affect growth and yield of grains: wheat [1–8], rice [1,8–10], sorghum [1], and maize [11–15]; and tubers: cassava [16] and potato [17–19]. Changes in growth temperature result in different whole-plant dry matter [15], mature grain weight [8], and starch properties [9–11,20]. Brown [12] reviewed maize environmental temperature responses and identified four major factors: (*a*) development rate was not a direct function of the temperature scale; (*b*) developmental response to temperature was not the same in all developmental subperiods nor was it the same for all cultivars; (*c*) other environmental variables needed to be considered in some developmental subperiods; and (*d*) temperature fluctuations, particularly diurnal range differences, might affect development rate, and the timing of the fluctuations might be very important.

Starch biosynthesis is directly affected by environmental temperature [21–24]. Temperatures higher than the optimum reduce the starch deposition rate in cereal grains [1,6,7] and potato tubers [18,19], and also shorten the duration of wheat and rice grain filling [8,25]. Because temperature changes affect starch biosynthesis, the structures of starch from different developmental temperatures differ. High temperatures significantly decrease the amylose content in rice [9] and high-amylose maize starch [11], and also change the fine structure of rice amylopectin [10]. Different developmental temperatures change starch structures and result in property differences. Starch quality variability necessitates continuous adjustments of many industrial processing parameters and carries over a potential quality control problem in various products.

The studies mentioned, except those of Bhullar and Jenner [6,7] on wheat, involved temperature and other environmental variable effects on whole plants. The objective of this work was to reveal, by minimizing other environmental variables, effects of developmental temperature of maize ear on its grain quality and starch fine structure.

2. Experimental

Materials.—The following materials were purchased and used without modification: Spectra/Mesh macroporous 53 μm nylon filters (Spectrum, Los Angeles, CA); amyloglucosidase (EC 3.2.1.3) from *Rhizopus* mold (Sigma Chemical Co., St. Louis, MO); Glucose Diagnostic Kit 115-A (Sigma Chemical Co., St. Louis, MO); Isoamylase (EC 3.2.1.68) from *Pseudomonas amyloderamosa* ATCC 21262 (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan); Sepharose CL-2B gel (Pharmacia Inc., Piscataway, NJ); and Bio-Gel P-6 gel (Bio-Rad Laboratories, Hercules, CA).

Production of starch at different developmental temperatures.—Two genetically unrelated normal dent maize inbreds, ICI63 and ICI93, with different heterotic backgrounds were produced in the Research Department of ICI Seeds, Slater, IA. Ten maize plants of each variety were grown in an environmentally controlled greenhouse with a temperature of 20–25 °C and a photoperiod of 15 h. There was one ear on each maize

plant. Fourteen days after pollination, the ears of four plants were wrapped with heating mantles and a thermocouple was inserted between each developing kernel and the husk in order to control the ear developmental temperature at 35 °C until maturity, as defined by the black layer formation. The ears not wrapped with heating mantles developed at greenhouse temperature (ca. 25 °C). The post-pollination waiting-period of 14 days was to avoid adverse effects on ear development [3,9]. Grain from six ears at 25 °C was collected, bulked together, and designated as the '25 °C sample'; grain from each of four heated (35 °C) ears was maintained separately for kernel dry weight and kernel density determination, bulked together for other analyses, and designated as the '35 °C sample'. The '25 °C sample' group was used as the control because the greatest maize yields are associated with daytime maximums of 24–30 °C [26].

Determination of kernel dry weight and kernel density.—Kernel dry weight was measured on six subsamples of 10 kernels each for the bulked 25 °C grown kernels, and duplicate subsamples of 10 kernels each for the four heated (35 °C) ears. The kernels were dried in a forced-air oven at 80 °C for 48 h before the measurement was made. To adjust moisture for kernel density determination, the kernels were equilibrated to approximately 12% moisture in a temperature–humidity-controlled incubator at 27 °C and 67% relative humidity. Kernel density was measured by using a Multi-Pycnometer Model MVP-1 (Quantachrome Crop., Syosset, NY) with nitrogen gas.

Determination of proximate chemical composition.—For determination of proximate chemical composition, maize kernels were ground by using a coffee mill (Model KSM2, Braun, Lynnfield, MA). Kernel moisture was determined by following the modified vacuum-oven method, AACC Approved Methods of Analysis 44-40 [27]. Protein content was determined by following the Kjeldahl method, AACC Approved Methods of Analysis 46-12 [27], and 6.25 was used as the nitrogen–protein converting factor. Kernel starch content was measured by following the AACC Approved Methods of Analysis 76-11 [27] with modifications in which the glucose content of the hydrolysate was analyzed by using the Sigma Diagnostics Kit for Determination of Glucose 115-A [28].

Starch isolation and fractionation.—Starch from four samples (two varieties at two developmental temperatures) was isolated in the laboratory by following the methods of Badenhuizen [29] with minor modifications. The kernels were soaked in 0.01 M $HgCl_2$ solution for 2 days to soften the kernels and to prohibit the activity of kernel amylase. The soaked kernels were blended by using a Hamilton Beach blender, Model 585-1 (Hamilton Beach Inc., Washington, NC), with two parts of 0.01 M $HgCl_2$ solution for 3 min. The germ and fiber residues were removed by filtering through a gauze and a 53-μm nylon Spectra/Mesh macroporous filter. The protein was removed from the starch slurry by using a 0.1 M NaCl solution with saturated toluene. Starch was collected by using a higher relative centrifugal force (3500 g, 20 min) than described by Badenhuizen [29], in order to prevent loss of small starch granules.

Fractionation of amylose and amylopectin was carried out by following the general procedure of Schoch [30] with slight modifications [31]. The recrystallization procedures for purifying amylopectin and amylose were repeated four times.

Determination of starch granule size.—The isolated starch was mounted on a glass microscope slide and viewed with a Zeiss axiophot microscope (Zeiss-Kontron, Thorn-

wood, NY) at 50 × magnification (20 × by 2.5 × optivar). Images of the starch granules were obtained by following the procedure described by Jane et al. [32].

Starch X-ray diffraction pattern.—Starch samples were moistened by equilibrating them in a saturated relative humidity chamber for 1 day at room temperature. Starch X-ray diffraction was performed on a Siemens D-500 X-ray diffractometer (Siemens, Madison, WI) with Cu $K\alpha$ radiation. The signal of reflection angle, 2θ, from 4 to 40 degrees, was recorded. Other operations followed procedures described elsewhere [32,33].

Determination of starch thermal properties.—Starch–water suspension (30%) was sealed in an aluminum pan (Perkin–Elmer, Norwalk, CT) and allowed to equilibrate at room temperature for 2 h before each analysis. The gelatinization temperature and enthalpy change of starch were determined by using a differential scanning calorimeter (DSC-7, Perkin–Elmer, Norwalk, CT) following the procedure of Jane et al. [32]. An identical empty aluminum pan was used as the reference.

Determination of iodine affinity of starch components and amylose content.—The iodine affinities of amylose, amylopectin, and defatted starch [34] were determined by an automatic potentiometric titrator (702 SM Titrino, Metrohm, Herisau, Switzerland) following the procedure of Schoch [35]. Data were recorded by using Metrodata software (Vesuv 2.0, Metrohm, Herisau, Switzerland) on an IBM computer. The amylose content was calculated following the method of Takeda et al. [36]. The measurement deviation of amylose content was calculated from standard deviations of starch, amylopectin, and amylose [37], and statistical comparison was also performed [38]. Apparent amylose content was calculated by dividing the iodine affinity of the starch by 19.0%, the typical value of iodine affinity for purified maize amylose [35].

Starch components profile.—The starch solution was prepared by dispersing starch in a 90% Me_2SO solution, heating the solution in a boiling water bath (96 °C) for 30 min, stirring at room temperature overnight, precipitating and washing the starch precipitate with methanol, redissolving it in hot water, and filtering the solution through a Whatman No. 52 filter paper. Starch solution (5 mL) containing 15 mg of starch was injected into a GPC column packed with Sepharose CL-2B gel (2.6 i.d. × 90 cm). The eluant containing 1 mM NaOH and 25 mM NaCl was applied in an ascending direction with a flow rate of 30 mL/h, and fractions of 4.6 mL each were collected and analyzed for total carbohydrate and iodine-staining blue value by following the procedure of Jane and Chen [31].

Molecular structure of amylopectin.—The amylopectin was debranched with *Pseudomonas* isoamylase [39]. The debranched sample was filtered through a 0.45-μm nylon syringe filter (Alltech Associates, Deerfield, IL). The branch-chain length distribution profile of amylopectin was determined by using a Bio-Gel P-6 GPC column (1.5 i.d. × 95 cm). After the injection of 2 mL of hydrolysate containing 10 mg of debranched amylopectin, samples were eluted with water in a descending direction with a flow rate of 30 mL/h. Fractions of 2 mL each were collected and analyzed for carbohydrate concentration by an anthrone–H_2SO_4 method [31,40] and reducing sugar concentration by a modified Park–Johnson method [31,41]. The peak chain-length was calculated by dividing the total carbohydrate concentration by reducing sugar concentration.

Chain-length distribution of amylopectin was also performed by using high-perfor-

Table 1
Kernel dry weight and kernel density differences by variety and temperature

Sample		Kernel dry weight [a] (mg/kernel)	Kernel density [b] (g/mL)
ICI63	25 °C	179.1 ± 2.4	1.323 ± 0.001
	35 °C	155.7 ± 2.3	1.211 ± 0.007
ICI92	25 °C	238.5 ± 2.0	1.299 ± 0.001
	35 °C	148.0 ± 1.7	1.168 ± 0.114

[a] Means from six subsamples of 10 kernels each for 25 °C grown kernels and duplicate subsamples of 10 kernels each of four 35 °C grown ears.
[b] Means from three subsamples for 25 °C grown kernels and one sample each of four different 35 °C grown ears.

mance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Dionex, Sunnyvale, CA) equipped with a DX-300 gradient chromatography system, a Dionex pulsed amperometric detector, a CarboPac PA1 (4 × 250 mm) column, and a CarboPac PA guard column (3 × 25 mm) (Dionex). A sodium nitrate gradient was applied as described by Wong and Jane [42].

Statistical analysis.—Data were analyzed by using Student's *t*-test in a general linear model (GLM) procedure on an SAS system (release 6.06, SAS Institute, Cary, NC). Means, standard deviations, and significant levels were calculated.

3. Results and discussion

To avoid temperature effects on other parts of the plants (e.g., roots and leaves), a heating mantle was used to wrap only each individual maize ear in order to control the developmental temperature of starch. Results showed that kernel dry weight developed at 25 °C was greater than that at 35 °C (Table 1). The effect of temperature on kernel dry weight was more intense for ICI92 (37.9% decrease) than ICI63 (13.1% decrease) when the temperature increased from 25 to 35 °C. Additionally, at 25 °C developing temperature, kernel dry weight of ICI92 was 24.9% greater than that of ICI63. At 35 °C, however, the weight of ICI63 was 5.2% greater than ICI92.

Kernels developed at 25 °C had a greater density than those at 35 °C. When the temperature increased from 25 to 35 °C, ICI92 suffered a greater kernel density loss (10.08%) than ICI63 (8.47%). This kernel density loss is an important quality concern because neither of the kernels grown at 35 °C meet the criteria of kernel density for dry milling food-grade maize in which the major grit fraction must be greater than 1.27 g/mL [26].

Table 2 shows the proximate chemical compositions of the maize kernels. Moisture contents of these samples were 12 to 13%; the protein contents were ca. 12%. ICI63 had greater starch contents (ca. 70%) than ICI92 (64.1 and 62.1% for 25 and 35 °C, respectively). Temperature variation did not significantly affect the proximate chemical compositions of the kernels. From the kernel dry weight and proximate composition data, the total dry matter of the maize kernel decreased as the developmental temperature increased; ICI92 suffered severer dry-matter loss than ICI63.

Table 2
Proximate composition of maize kernel from different developmental temperatures [a]

Sample		Protein [b] (%)	Starch [b] (%)	Moisture (%)
ICI63	25 °C	12.4 ± 0.4	69.3 ± 1.6	12.38 ± 0.12
	35 °C	11.8 ± 0.1	70.1 ± 1.5	12.03 ± 0.08
ICI92	25 °C	12.2 ± 0.3	64.1 ± 1.6	13.73 ± 0.10
	35 °C	12.6 ± 0.2	62.1 ± 2.0	13.07 ± 0.09

[a] Means and standard deviations from three subsamples.
[b] On dry basis.

ICI63 had greater dispersity of granule size distribution than did ICI92 (Table 3). Both ICI63 and ICI92 grown at 35 °C had greater populations of small granules. The number-average starch granule size of ICI63 decreased from 11.96 to 10.45 μm, whereas the average of ICI92 decreased from 10.78 to 10.33 μm as the temperature increased from 25 to 35 °C (Table 3). The length/width (L/W) index, the value of maximum diameter divided by minimum diameter, was used as an index of the starch granule shape. L/W values equal 1 for the perfect round shape. The average L/W index of ICI63 increased from 1.19 to 1.24, which indicated more oval-shaped starch granules as the temperature increased from 25 to 35 °C; there was no effect found on the L/W index of ICI92. The data support the early observation of Badenhuizen [43] that smaller and more abnormal-shaped starch granules are found in waxy maize starch developed at 30 °C incubation than those at 24 °C. The results also showed that the impact of the developing temperature on starch granule shape varied with different varieties.

X-ray diffractograms of both starch varieties at 25 and 35 °C were similar, and all showed a typical A pattern (Fig. 1). The thermal properties of maize starch, however, varied with the variety and the developmental temperature change. ICI63 had a higher

Table 3
Granular size of maize starch from different developmental temperatures [a,b]

Sample		Diameter of equivalent circle (μm) [c]		Length/width index [d]	
		Average	Range	Average	Range
ICI63	25 °C	11.96 ± 3.92 *	1.27–22.68	1.19 ± 0.15 †	1.00–2.03
	35 °C	10.45 ± 4.47 †,‡	0.20–25.34	1.24 ± 0.23 *	1.00–3.26
ICI92	25 °C	10.78 ± 3.18 †	2.35–19.64	1.15 ± 0.16 ‡	1.00–2.43
	35 °C	10.33 ± 3.20 ‡	1.21–18.49	1.15 ± 0.13 ‡	1.00–1.80

[a] Data from 690 starch granules.
[b] Figures in the same column having the same superscript are not significantly different ($P < 0.05$).
[c] Calculated from an area assumed to be a perfect circle.
[d] Calculated by dividing maximum diameter by minimum diameter.

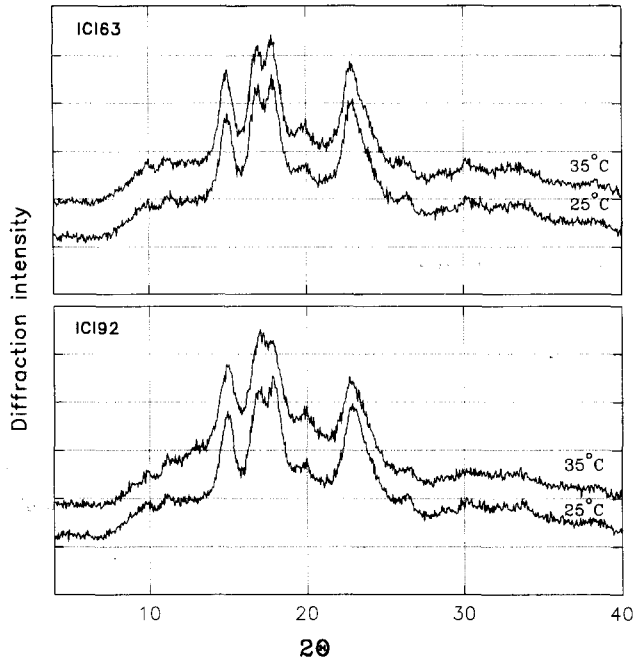

Fig. 1. X-ray diffractograms of maize starches developed at different temperatures. The signals were recorded for diffraction angle (2θ) from 4 to 40 degrees.

onset gelatinization temperature (T_o) and a narrower temperature range ($T_c - T_o$) than ICI92 (Table 4). The starch developed at 35 °C had a higher gelatinization temperature and wider temperature range than that at 25 °C, and the effect was more significant on ICI63 than on ICI92. When the developmental temperature increased from 25 to 35 °C, onset gelatinization temperatures of ICI63 and ICI92 increased 2.4 and 1.8 °C, respectively. This observation was consistent with those reported for rice starch [9] and for wheat starch [20]. The gelatinization enthalpy was not significantly affected by the

Table 4
Thermal properties of maize starch from different developmental temperatures [a,b]

Sample		T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	Enthalpy (J/g)
ICI63	25 °C	69.1±0.3	72.0±0.3	82.1±1.4	13.0±1.4	14.3±1.0
	35 °C	71.5±0.2	74.9±0.2	87.5±2.1	16.0±2.1	15.2±0.6
ICI92	25 °C	66.8±0.4	73.0±0.4	83.4±1.3	16.6±0.9	14.1±1.5
	35 °C	68.6±0.6	73.8±0.1	86.3±1.7	17.6±2.0	13.7±1.2

[a] Means and standard deviations from six measurements.
[b] T_o: Onset temperature; T_p: peak temperature; T_c: conclusion temperature.

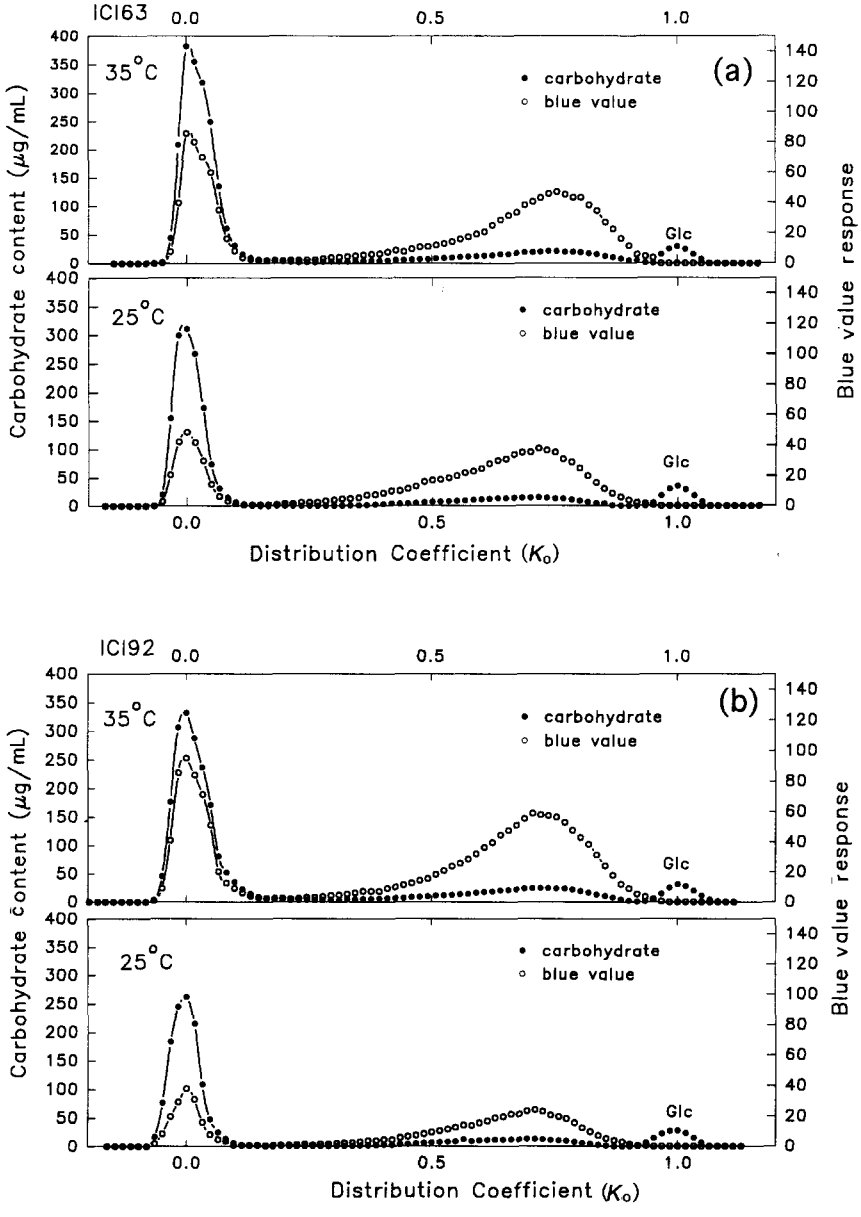

Fig. 2. Sepharose CL-2B chromatograms of ICI maize starches developed at different temperatures. Glc: glucose was used as the marker.

elevated developmental temperature. These results indicated that starch thermal properties varied with both genetic background and developmental temperature.

Sepharose CL-2B gel permeation chromatograms (Fig. 2) show the molecular size distribution of starch. Distribution coefficients [44], K_o, instead of elution volumes,

Table 5
Iodine affinity of maize starch and its components from different developmental temperatures [a,b]

Sample		IA_{starch}	$IA_{amylose}$	$IA_{amylopectin}$	Apparent amylose (%) [c]	Amylose (%) [d]
ICI63	25 °C	5.35 ± 0.01	19.29 ± 0.15	1.56 ± 0.13	28.16 ± 0.06	21.37 ± 0.80 [e]
	35 °C	4.82 ± 0.06	19.37 ± 0.08	1.41 ± 0.01	25.35 ± 0.31	18.98 ± 0.35
ICI92	25 °C	5.27 ± 0.05	18.66 ± 0.03	1.82 ± 0.04	27.74 ± 0.25	20.51 ± 0.38
	35 °C	5.19 ± 0.06	18.81 ± 0.19	2.14 ± 0.02	27.34 ± 0.30	18.31 ± 0.42

[a] Means and standard deviations from three measurements.
[b] IA: iodine affinity (g/100 g).
[c] Calculated from $(IA_{starch}/IA_{amylose}) \times 100$, where $IA_{amylose}$ was assumed to be 19.0.
[d] Calculated from $[(IA_{starch} - IA_{amylopectin})/(IA_{amylose} - IA_{amylopectin})] \times 100$.
[e] Deviations calculated from standard deviations of starch, amylopectin, and amylose; the estimated variance was used for Student's *t*-test.

were used for comparison between samples. The peak retention volume of amylopectin, the first peak of the profile, was used as void volume, $K_0 = 0$, and the peak retention volume of glucose, the third peak, was used as total permeation volume, $K_0 = 1$. As the developmental temperature increased from 25 to 35 °C, both K_0 values of amylose shifted to greater values (0.716 ± 0.002 to 0.754 ± 0.001 and 0.710 ± 0.001 to 0.736 ± 0.003 for ICI63 and ICI92, respectively), which indicated smaller molecular size for amylose.

Table 5 shows the iodine affinities of starch and its components. As the developmental temperature increased, iodine affinities and apparent and true amylose contents of both starch varieties decreased. The results of starch iodine affinities and apparent amylose contents were in agreement with Asaoka et al. for rice [9,10], and Fergason and Zuber for maize [11]; however, Shi et al. [20] found that the apparent amylose content of wheat increased with increase of growing temperature. The true amylose content result was consistent with that of rice starch [9,10]. The amylose iodine affinities of ICI63 were higher than those of ICI92; however, the amylopectin iodine affinities of ICI63 were less than for ICI92. When the developmental tempeature increased to 35 °C, amylose iodine affinities of both varieties increased, whereas the size of amylose molecules decreased. A possible reason for this phenomenon is that the smaller amylose molecules may be less branched [41]. The results indicated that iodine affinities of starch and its components varied with variety and developmental temperature.

The chain-length distributions of debranched ICI63 and ICI92 amylopectins by Bio-Gel P6 gel permeation chromatography are shown in Fig. 3. The elution profiles showed three peaks which were divided into three fractions, F1–F3, in the order of elution. The peak chain-lengths and the percentage of each fraction of those samples are summarized in Table 6. The first fraction of the chromatogram was a long branch-chain fraction, similar to that reported for non-waxy rice amylopectin [31,36,45,46] and for sugary and normal maize [47] as B3 and longer chains that stretched across three or more clusters in the amylopectin molecules [48]. The debranching reaction of each sample was replicated four times. Thus, we believed there was a minimal content of incompletely debranched molecules in the F1 fraction. The second peak, F2, was the

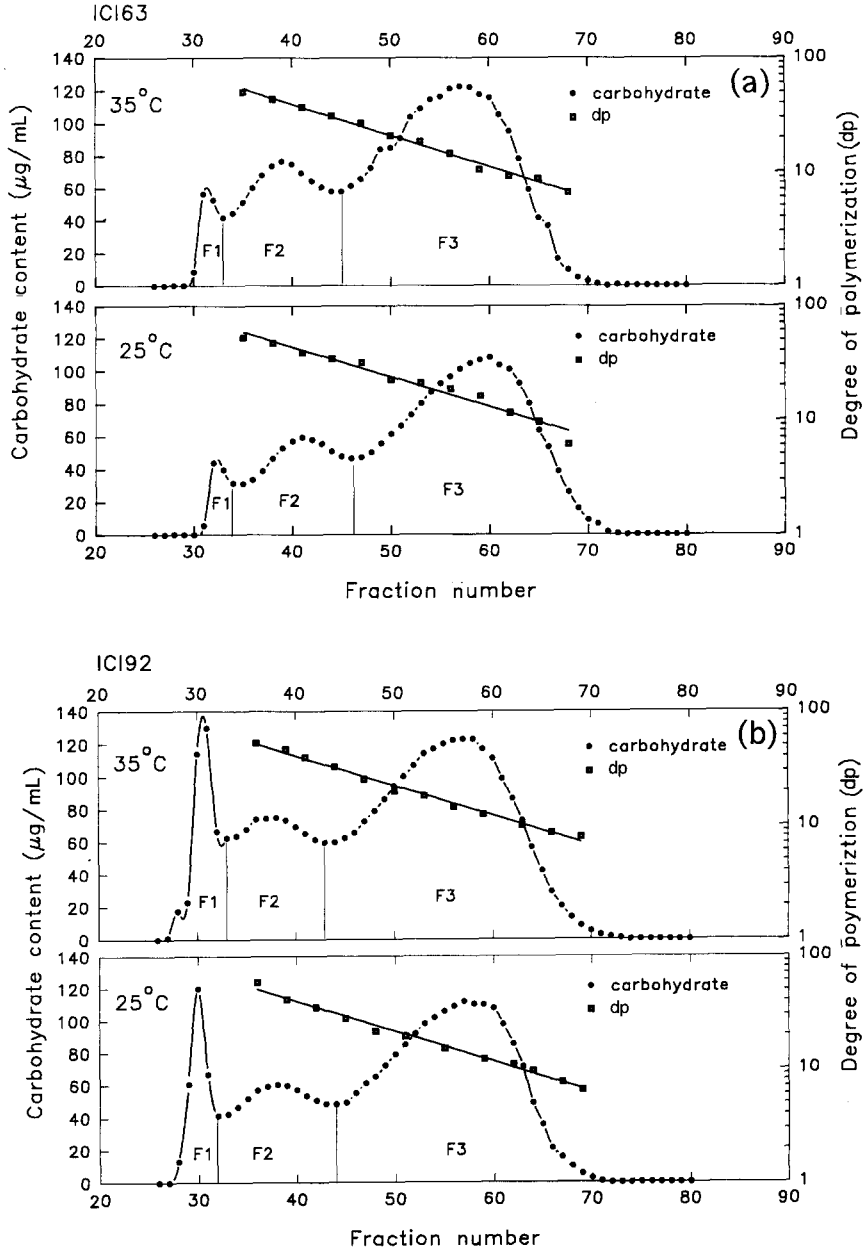

Fig. 3. Bio-Gel P6 chromatogram of isoamylase-debranched ICI maize amylopectin.

medium branch-chain (B2) fraction that stretched across two clusters in the amylopectin molecules. The third peak, F3, of the chromatograms was the short branch-chain (B1 and A) fraction.

Table 6
Temperature effect on branch-chain of maize amylopectin [a,b]

Sample		Peak chain-length		Weight percentage		
		F2	F3	F1	F2	F3
ICI63	25 °C	39.9±3.5	12.9±0.5	6.8±1.5	22.3±0.3	70.8±1.8
	35 °C	42.1±2.9	14.0±0.6	5.8±1.4	25.1±0.7	69.1±0.6
ICI92	25 °C	38.7±2.9	13.5±0.7	7.7±1.5	20.3±0.2	72.0±3.3
	35 °C	41.9±2.6	13.1±0.7	8.1±2.2	21.8±1.5	70.1±2.7

[a] Means and standard deviations from three or four measurements.
[b] The fractions, F1 to F3, correspond to Fig. 3.

The chain lengths and proportions of amylopectin branch-chain fractions varied with both variety and developmental temperature. The chain length of F2 increased as the developmental temperature increased, but the chain length of F3 showed no significant difference. The increase in chain length of F2 correlates with the increase of onset gelatinization temperature (Table 4). These results agree with those reported for rice starches [9,10] and for taro starch [32]. The proportions of long branch-chain, F1, of ICI63 were less than those of ICI92. The content of F1 correlates with the iodine affinity of amylopectin [36,46]. The proportions of F2 fraction increased as the developmental temperature increased. These results are in agreement with those for the temperature effect on rice starch [9,10]. The variety and developmental temperature effects on amylopectin structure were verified by studies of their physicochemical properties and HPAEC-PAD analysis.

Because Bio-Gel P6 can only reveal an overall view of length distribution of amylopectin branches, HPAEC-PAD was employed to show the individual components of each branch of amylopectin. Because the PAD detector response decreases when the branch-chain length increases, there was no quantitative result obtained in each individual profile. Direct comparisons between normalized detector response profiles were used to compare branch-chain length distributions, and the sum of the detector response from dp = 5 to 64 was used to normalize the profiles. The relative branch-chain length distribution of amylopectin is shown in Fig. 4. ICI63 amylopectin developed at 35 °C had a greater concentration between dp = 19 to 36 and a lesser concentration of dp = 7 to 16 and 41 to 49 than that developed at 25 °C. According to the modified cluster model proposed by Hizukuri [48], this increased portion can be cataloged as long B1 and short B2 chains. Meanwhile, the branch chains of ICI92 developed at 35 °C had a greater concentration between dp = 43 to 62 (equivalent to the B2 and B3 chains) and a lesser concentration of dp = 6 to 20 than that developed at 25 °C. These results indicated that the developmental temperature impact on maize amylopectin structure varied with the variety.

The biochemical mechanism explaining the increasing concentration of branches of dp 19 to 36 in ICI63 and dp 43 to 62 in ICI92 amylopectin structure is consistent with the reported effects of temperature on the enzymes of starch synthesis (starch synthases and branching enzymes) [23,49,50]. The precise enzymology of how starch is assembled

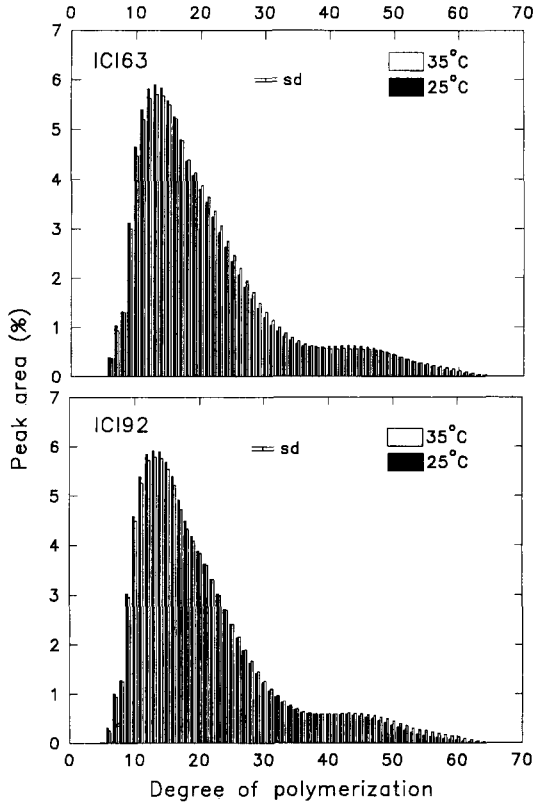

Fig. 4. Temperature effect on branch-chain length distribution of ICI maize amylopectin analyzed by HPAEC-PAD. Results are given as the mean of three measurements. The error bar marked with sd is the average standard deviation of all branch-chain components.

is not well understood; however, it is generally believed that amylopectin synthesis proceeds through the combination of chain elongation by soluble starch synthase, linked with inter-chain branching by branching enzyme. In a developing maize kernel there are multiple forms of starch branching enzyme (BE) and soluble starch synthase, which must interact together to form the complex, but ordered, fine structure of starch. The effects of elevated temperature on starch fine structure may be explained by the effects of temperature on the synthetic enzymes, or by temperature-induced changes in the starch substrate itself. Firstly, BEI, with a minor branching-enzyme activity [50], preferentially transfers long chains and has a temperature optimum of 35 °C [49], whereas BEIIa and BEIIb, with the major branching-enzyme activity that transfers short chains, have temperature optimums of 25 and 20 °C, respectively [49]. Secondly, the different isoforms of soluble starch synthases also show differences in sensitivity to elevated temperature [23]. Thirdly, branch-chain formation has been proposed to operate by transferring chains between double helices [51,52]. Elevated temperature decreases formation of the starch double helix but increases the chain length of the double helix. A

detailed study of the temperature effect on amylose retrogradation is reported elsewhere [53]. These factors alone or in combination may cause amylopectin developed at 35 °C to have more long branches than amylopectin developed at 25 °C.

In conclusion, two maizes, ICI63 and ICI92, with unrelated genetic backgrounds responded differently to developmental temperature change from 25 to 35 °C. At 35 °C, kernel weight and kernel density decreased, with ICI63 maintaining grain yield better than ICI92. The increased grain-developing temperature was responsible for changes in starch structure and property changes (increased small granules and gelatinization temperature, and decreased amylose content). ICI63 amylopectin had an increased medium branch-chain fraction and decreased long and short branch-chain fractions, whereas ICI92 had increased long and medium branch-chain fractions as the developmental temperature increased.

Acknowledgements

The authors thank ICI Seeds Company, Slater, IA for financial support.

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