Carbohydrate Polymers 51 (2003) 439–450

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Thermal and structural properties of unusual starches from developmental corn lines☆

Y. Ji[a], K. Seetharaman[a,b], K. Wong[a], L.M. Pollak[c], S. Duvick[c], J. Jane[a], P.J. White[a,*]

[a]*Department of Food Science and Human Nutrition, Iowa State University, 2312 Food Science Building, Ames, IA 50011, USA*
[b]*Department of Food Science, The Pennsylvania State University, University Park, PA 16802, USA*
[c]*USDA-ARS, Corn Insects and Crop Genetics Research Unit, Department of Agronomy, Iowa State University, Ames, IA 50011, USA*

Received 17 May 2002; revised 20 July 2002; accepted 23 July 2002

Abstract

Starches from exotic corn lines were screened by using differential scanning calorimetry (DSC) to find thermal properties that were significantly different from those exhibited by starches from normal Corn Belt lines. Two independent gelatinization transitions, one corresponding to the melting of a peak at ~66 °C and the other to a peak melting at ~69 °C, were found in some starches. The melting characteristics were traced to two separate types of granules within the endosperm. Strong correlations were found between DSC properties and proportion of large granules with equivalent diameter ≥ 17 μm. Starches with a lower peak onset gelatinization temperature (T_{oG}), had a lower normalized concentration of chains with a degree of polymerization (dp) of 15–24 and/or a greater normalized concentration of chains with a dp of 6–12. These studies will aid in understanding structure–thermal property relationships of starches, and in identifying corn lines of interest for commercial breeding.

Keywords: Maize starch; Corn starch; Starch structure; Starch function; Starch thermal properties; Starch granules; Starch molecular weight; Starch branch-chain-length

1. Introduction

Although corn starch is a valuable ingredient to the food industry, the cooking characteristics of native, unmodified corn starches are undesirable for many applications. To overcome these problems and expand the usefulness of starch, chemical modifications (such as cross-linking and/or substitution) and physical modifications (such as pregelatinization) often are made. With more restrictive food regulations and little hope for the introduction of new modification processes, a greater potential for the manufacture of modified starches with improved functionalities lies in the use of raw materials from previously uncharacterized plant genotypes. This approach might be especially valuable to the food industries, because the corn and its starch could be used in the manufacture of 'all natural' foods.

As the domestic gene pool becomes more genetically homogeneous, exotic (non-Corn Belt Dent) germplasm may be an excellent source for improving quality and, possibly, improved agronomic performance, because much of the corn grown outside of the US is consumed directly by humans and has undergone centuries of selection for flavors, aromas, and textures (Tracy, 1990). Exotic germplasm is usually considered to include unadapted domestic populations and foreign temperate, tropical, and semi-tropical populations. Geadelmann (1984) suggested that incorporation of exotic strains into adapted germplasm would increase the available genetic variability and give rise to additional heterotic vigor, lessening chances for a yield plateau. The introgression of adapted germplasm with useful genes from exotic corn has successfully altered corn traits and broadened the genetic base through the germplasm enhancement of maize (GEM) project (Pollak & Salhuana, 1999). The small amount of data that has been collected suggests the presence of significant variability in the thermal properties of starch from these corn genetic resources (Campbell, White, & Pollak, 1995; White, Abbas, Pollak, & Johnson, 1990).

☆ Journal Paper No. 19849 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project No. 3768, and supported by Hatch Act and State of Iowa funds.

* Corresponding author. Tel.: +1-515-294-9688; fax: +1-515-294-8181.
E-mail address: pjwhite@iastate.edu (P.J. White).

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PII: S0144-8617(02)00216-3

Table 1
Exotic breeding crosses and exotic inbred corn lines and their origins

Exotic parent[a]	Pedigree for S_n lines[b]	Source identification[c]	Number of S_{n+1}[d] lines analyzed	Number of unusual S_{n+1} lines[e]	Origin of exotic parent
Exotic breeding crosses					
PI 576258	CHIS775:S1911b-37-1-2	Chis-37	16	9	Mexico
PI 489361	CUBA164:S2008a-23-1	Cuba-23	1	1	Cuba
PI 489361	CUBA164:S1511b-34-1-3	Cuba-34	8	2	Cuba
PI 489361	CUBA164:S1511b-38-1-3	Cuba-38	8	2	Cuba
Ames 23670	DK212T:S0610-8-1-3	DK-8	5	3	Thailand
Ames 23670	DK212T:S0610-10-1-3	DK-10	21	3	Thailand
Exotic inbreds					
PI 186182	PI 186182	PI-82	8	4	Uruguay
PI 186183	PI 186183	PI-83	10	8	Uruguay

[a] Original corn populations as maintained at the North Central Region Plant Introduction Center, Ames, IA.
[b] Regrown corn ears, maintained as lines to preserve a specific starch characteristic. $n = 3$ for exotic breeding crosses except Cuba-23 for which $n = 2$. $n = 1$ for exotic inbreds.
[c] Abbreviated source identification for use within this paper, representing the S_n lines.
[d] S_{n+1} line means first generation of corn after self-pollination of S_n lines.
[e] Number of S_{n+1} lines having at least one kernel containing starch with unusual thermal properties according to criteria given by Seetharaman et al. (2001).

The genetic background of corn can have substantial effects on the physical and chemical properties of starch. This influence may be attributable to changes in granule-size distribution (Campbell, Li, Berke, & Glover, 1996; Katz, Furcsik, Tenbarge, Hauber, & Friedman, 1993), chemical structure (Lim, Kasemsuwan, & Jane, 1994), crystallinity (Stering, 1962), organization of the molecules within the granule (Shannon & Garwood, 1984), and/or molecular structure of the starch polymers (Jane et al., 1999; Sanders, Thompson, & Boyer, 1990).

We previously identified six novel corn lines, of which two are exotic germplasm lines, and four are derived from breeding crosses developed by crossing exotic genotypes with Corn Belt lines. These lines have starches with significantly different (and potentially useful) thermal properties from those found in starch from normal Corn Belt corn. The objective of this study was to evaluate the thermal properties of novel corn lines regrown and self-pollinated in 1998 and to establish the relationship between the fine structure and physicochemical properties of the starch.

2. Materials and methods

2.1. Corn populations

Lines and their advanced progeny from six exotic by adapted breeding crosses from the GEM project and two exotic Plant Introductions, plus public Corn Belt inbred lines B73 (Stiff Stalk heterotic pattern) and Mo17 (non-Stiff Stalk heterotic pattern) as controls, were studied (Table 1). The S_n designation defines the number of times the line has been self-pollinated, starting with the breeding cross (the S_0 population), in the development of the line (Simmonds, 1974). For example, an S_3 line has been self-pollinated three times after starting in the S_0 population. With each self-pollination, the numbers of heterozygous genes are reduced by half, thus increasing inbreeding, genetic purity, and repeatability of a trait when it is replanted. The original exotic lines and populations used in this study are maintained at the North Central Regional Plant Introduction Station in Ames, IA. The breeding crosses were developed by crossing the exotic populations with inbreds of the Stiff Stalk heterotic pattern. The Stiff Stalk inbreds belong to companies that cooperate in GEM (Pollak & Salhuana, 1999). All S_3 families for the exotic breeding crosses, S_1 families for the exotic inbreds, and B73 and Mo17 as controls were grown and self-pollinated in the same environment near Ames, IA, in 1998, to reduce the effect of differences caused by environment. Ears were harvested at full maturity and dried at 37.5 °C until the moisture content reached 12%. All seeds were stored at 4 °C and 10% relative humidity until analyzed.

2.2. Single-kernel starch extraction

Starch was extracted from single kernels using the method described by White et al. (1990), with modifications (Krieger, Duvick, Pollak, & White, 1997). For the initial screening, at least 10 randomly selected kernels from up to 21 progeny lines from each of the eight exotic families were individually evaluated for starch characteristics after extraction. Thermal analysis by using differential scanning calorimetry (DSC, described later) was conducted on these starch samples. Based on the results of the initial screening, 32 progeny lines from eight exotic families, plus one line each from Mo17 and B73 as controls, were selected for further characterization. After extraction, starch was stored at 4 °C until evaluated.

2.3. DSC

A Perkin–Elmer DSC-7 analyzer (Norwalk, CT), equipped with thermal analysis software (Perkin–Elmer Corporation, Norwalk, CT), was used to analyze starch thermal properties following procedures of White et al. (1990). All experiments were run at a scanning rate of 10 °C/min from 30 to 110 °C, and samples were prepared using a water-to-starch ratio of 2:1. The actual dry weight of starch used ranged from 3.96 to 4.02 mg. DSC parameters recorded for this study included change in enthalpy (ΔH), peak onset (T_o), peak (T_p), and range of gelatinization (R_G). A subscript G after the parameter denotes a gelatinization property. The parameters T_o, T_p, T_c (peak end), and ΔH were given directly by the DSC software. The R_G was calculated as $T_c - T_o$, and peak height index (PHI) was calculated from the change in enthalpy of gelatinization divided by half the range. All enthalpy calculations were based on the dry-starch weight, and all analyses were conducted in duplicate and values averaged. The same scanning method was used for retrogradation of the gelatinized samples kept at 4 °C at 7 days. A subscript R after the DSC parameter denotes a retrogradation property.

2.4. Microscopy analyses

Birefringence of individual starch granules was evaluated with a polarized microscope equipped with cross-polarizers at 40 × magnification (Nikon, Japan). To prepare the partly gelatinized starch sample, 33% water suspensions of a starch sample were sealed in an aluminum DSC pan and heated to 66 °C (the temperature between two gelatinization peak maxima previously identified on the DSC thermograms of some starches) at a heating rate of 10 °C/min, and then cooled in an ice bath. The sealed DSC pan was opened with tweezers, and the partly gelatinized starch was removed and applied to a glass microscope slide with a drop of mineral oil added to enhance the vision. The presence of ungelatinized starch granules was determined.

Granule-size distributions of native starches were obtained by following the procedure described by Jane and Chen (1992). Native starch was suspended in 100% ethanol to aid in spreading the particles into a monolayer, mounted on a glass microscope slide, and viewed using a Zeiss axiophot microscope (Zeiss-Kontron, Thornwood, NY) at 50 × magnification (20 × by 2.5 × optivar). Three slides from each sample were analyzed separately, with 200 particles measured from one slide and 400 particles measured from each of two additional slides, to give a total of 1000 starch granules analyzed per starch type. The starch granules selected for the measurement were randomly chosen from each slide. For each granule, area, perimeter, radial SD, and major axis were determined. The radial SD is a measure of the 'roundness' of a particle. A perfect circle would have a radial SD measurement of 0. The less round the particle, the bigger its radial SD number. The equivalent diameter was assessed by: equivalent diameter = sqrt(4 × area/π).

2.5. Molecular size of amylose

A starch components profile, indicating the molecular size of amylose, was determined by gel-permeation chromatography on a sepharose CL-2B column (Jane & Chen, 1992). Distribution coefficients, K_0, of amylose were used for comparison between samples. The peak retention volume of amylopectin was used as the void volume, $K_0 = 0$, and the peak retention volume of glucose was used as the total permeation volume, $K_0 = 1$. The results are the average of three replicate analyses of each starch type.

2.6. X-ray diffraction

The X-ray diffraction patterns of starches were obtained with copper–nickel foil-filtered, Kα radiation by using a diffractometer (D-500, Siemens, Madison, WI) following the procedure described by Jane et al. (1999). The diffractometer was operated at 27 mA and 50 kV. The scanning region of the diffraction angle (2θ) was from 4 to 40° at 0.05° increments with a count time of 2 s.

2.7. Branch-chain-length distribution of whole starch

Branch-chain-length distribution of starch was determined following the procedure described by Jane and Chen (1992). Starch extracted as previously described for single-kernel extracting was debranched by using isoamylase, and the branch-chain-length distributions were analyzed by using a high-performance anion-exchange chromatography system equipped with an enzyme column reactor and a pulsed amperometric detector (Dionex, Sunnyvale, CA) (HPAEC-ENZ-PAD) by using the method reported by Wong and Jane (1997). The results reported are an average of at least two replicates for each sample.

2.8. Statistical analysis

Granule size and shape distribution of starches were analyzed by using software entitled S-plus 6 (Insightful Corporation, Seattle, WA). Between sample variations of granule size and shape parameters, which included mean area, equivalent diameter, perimeter, radial SD, and major axis, were assessed by using the mixed effects Analysis of Variance (ANOVA) model for nested design (i.e. three plates were nested within a starch sample) with unbalanced replicates. The Tukey multiple comparison test was used to calculate differences in means of these parameters among starch samples. Relationships between starch DSC properties and granule size and shape distribution were analyzed by using the Pearson correlation test in the SAS system (release 8.2, SAS Institute, Cary, NC). The coefficients (K_0) of amylose were analyzed by using the general linear model

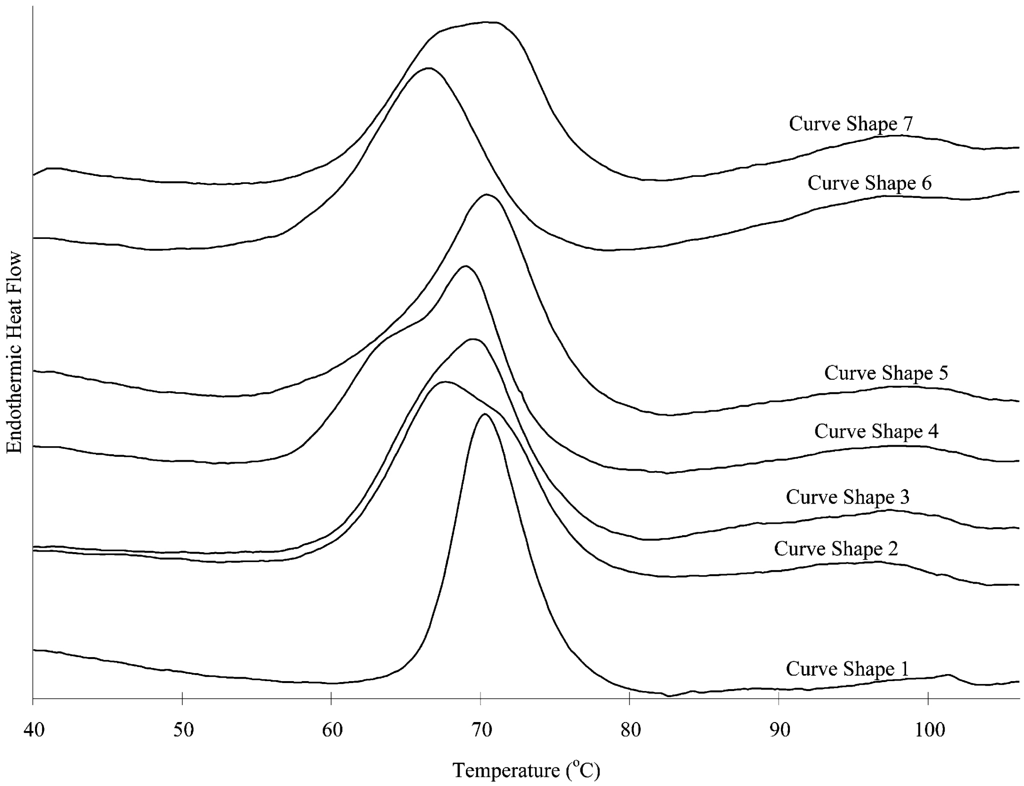


Fig. 1. Typical DSC gelatinization thermograms of starches listed in Table 3, illustrating the different curve shapes.

(GLM) procedure on the SAS system. Multiple comparison procedures of the Tukey test were used to calculate the differences among starch samples. The family wise confidence level used for calculating the differences among starch samples was 95% (i.e. $\alpha = 5\%$).

3. Results and discussion

3.1. DSC

Starch from 77 progeny S_{n+1} lines of the eight exotic breeding crosses and exotic inbred S_n families (1–21 S_{n+1} lines per S_n family) and two Corn Belt inbred lines used as controls (Mo17 and B73) were screened by using DSC to identify unusual thermal properties. A starch was considered to have different and useful thermal properties based on criteria given by Seetharaman et al. (2001). Each of eight exotic S_n families (Table 1) had at least one progeny S_{n+1} line whose kernels contained starch exhibiting one or more useful DSC properties. For example, 9 out of 16 progeny lines of the family Chis-37 that we analyzed contained at least one kernel having starch with a specific useful DSC property.

The frequency of kernels within each S_{n+1} line with starch exhibiting a specific DSC property varied from 1/10 to 36/37 (data not shown). Progeny lines from Chis-37, Cuba-23, Cuba-34, Cuba-38, and PI-83 had a high frequency of kernels (7/10 to 36/37) with both low T_{oG} (56.6–61.6 °C) and wide R_G (13.6–17.8 °C). Among them, 7 of 10 kernels of one progeny line from Chis-37 and 8 of 10 kernels of one progeny line from Cuba-38 also contained starches with low T_{pG} (66.1–66.2 °C). All selected starches (except one progeny line from Cuba-23) had a relatively lower ΔH_G (9.2–11.9 J/g) than did Mo17 (12.4 J/g). None of the starches exhibited unusual retrogradation properties.

Some starches from exotic lines also exhibited gelatinization thermogram shapes with shoulders or double peaks (Fig. 1), suggesting either two different independent cooperative transitions, or one time-dependent two-stage process during gelatinization of starches. To distinguish between these possibilities, a two-stage heating experiment was used. The DSC gelatinization curve for starch from one kernel of Cuba-23-1-12 is shown in Fig. 2(a), demonstrating the double peak with a large shoulder at the lower temperature. During the experiment, another sample of the starch suspension was heated to 66 °C, just beyond the peak temperature of the first peak (Fig. 2(b)), cooled, and then reheated beyond the gelatinization temperature of the second peak (Fig. 2(c)). During the second complete heating (Fig. 2(c)), only a small single peak was found. The position of the peak in Fig. 2(c) was similar to that of the second peak of the original gelatinization curve (Fig. 2(a)). This finding clearly indicated that the original curve of gelatinization represents two independent transitions, one corresponding to the melting of the lower temperature peak ($T_p \sim 66$ °C) and the other to a higher temperature peak ($T_p \sim 69$ °C). Different ratios of the two transitions may

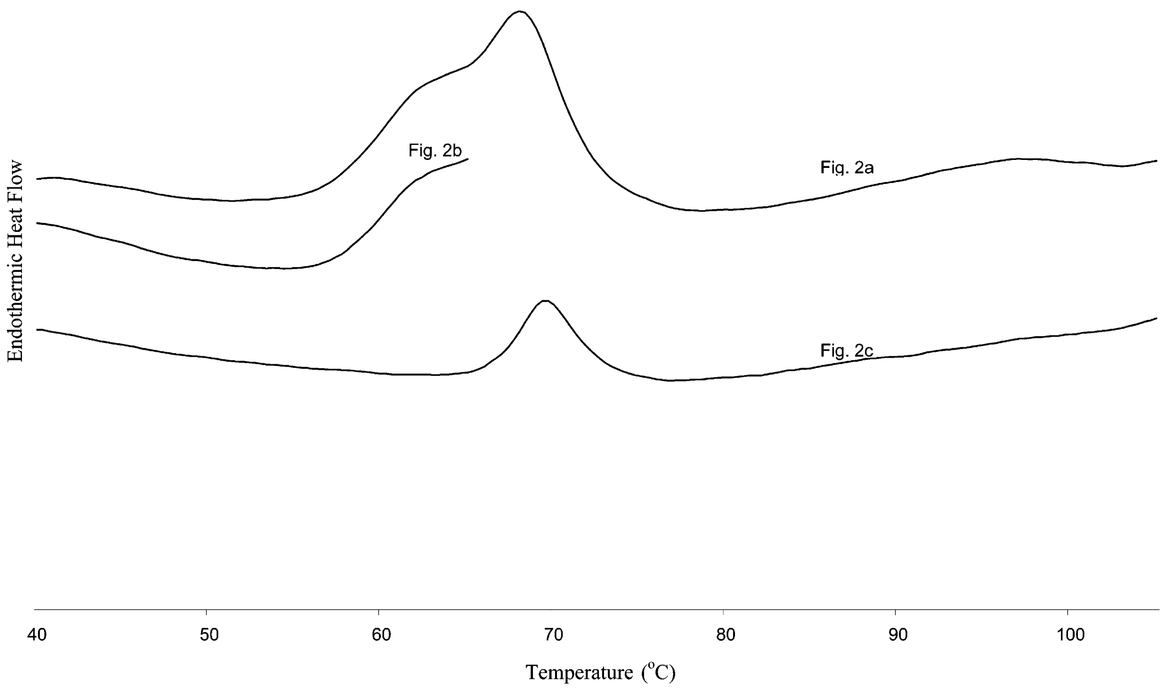


Fig. 2. DSC gelatinization thermograms of Cuba-23-1-12 starch. (a) Sample was heated from 30 to 110 °C. (b) Starch was heated from 30 to 66 °C (just after the temperature of the first peak). (c) Starch from (b) was cooled to 30 °C and then heated to 110 °C.

cause the different shapes of gelatinization peaks shown in Fig. 1.

Two transitions for these starches still existed when the DSC was performed in excess water. No significant differences in gelatinization parameters were observed when the starch/water ratio was increased from 1:2 to 1:4 for two different starches with different curve shapes, Cuba-23-1-12 and PI-83-9-5 (Fig. 3(a) and (b)). This observation

Table 2
DSC data of starches from single kernels of selected corn lines used in structural analysis

Source of starch (S_{n+1} line[a]-kernel)	DSC parameter							
	T_{oG} (°C)	T_{pG} (°C)	ΔH_G (J/g)	R_G (°C)	PHI	ΔH_R (J/g)	R (%)	Shape of DSC gelatinization curve[b]
Mo17	66.2	70.8	12.6	9.2	2.7	5.6	44.5	Curve shape 1
B73	67.2	71.1	11.6	7.6	3.1	6.0	51.6	Curve shape 1
Chis-37-5-3	**60.3**	**66.5**	10.5	**14.0**	1.5	5.7	54.5	Curve shape 2
Chis-37-11-1	62.1	70.6	9.3	**13.5**	1.4	3.6	38.6	Curve shape 3
Cuba-23-1-12	**58.4**	70.1	10.3	**18.3**	1.1	4.7	45.8	Curve shape 4
Cuba-34-1-2	**60.9**	70.4	11.7	**16.0**	1.5	4.5	38.0	Curve shape 5
Cuba-38-5-5	**57.9**	**66.1**	9.1	**16.3**	1.1	4.9	54.6	Curve shape 6
DK-10-1-2	63.2	71.6	11.3	**13.7**	1.6	6.3	56.3	Curve shape 3
PI-82-1-1	**60.4**	69.4	10.5	**15.1**	1.4	6.3	59.9	Curve shape 3
PI-83-2-2	**59.6**	69.9	10.8	**16.1**	1.3	5.6	51.4	Curve shape 3
PI-83-2-5	**60.4**	69.1	10.5	**16.0**	1.3	5.4	51.6	Curve shape 2
PI-83-2-10	**60.5**	67.6	10.4	**15.3**	1.4	5.5	52.6	Curve shape 2
PI-83-9-5	**60.6**	70.3	9.6	**15.8**	1.2	4.9	51.3	Curve shape 7
PI-83-9-8	**59.4**	71.2	9.3	**16.5**	1.1	4.6	49.9	Curve shape 3
PI-83-9-11	**58.9**	70.7	11.0	**17.2**	1.3	5.9	53.2	Curve shape 3

T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; R_G, range of gelatinization temperature; ΔH_G, enthalpy of gelatinization; ΔH_R, enthalpy of retrogradation; PHI, peak height index (enthalpy of gelatinization divided by half the range); R, retrogradation. Bold numbers indicate values of interest.

[a] S_{n+1} line means first generation of corn after self-pollination of S_n lines. For example, Chis-37-5 designates the S_{n+1} line. The −3 designates the kernel number.

[b] See Fig. 1 for definition of curve shapes.

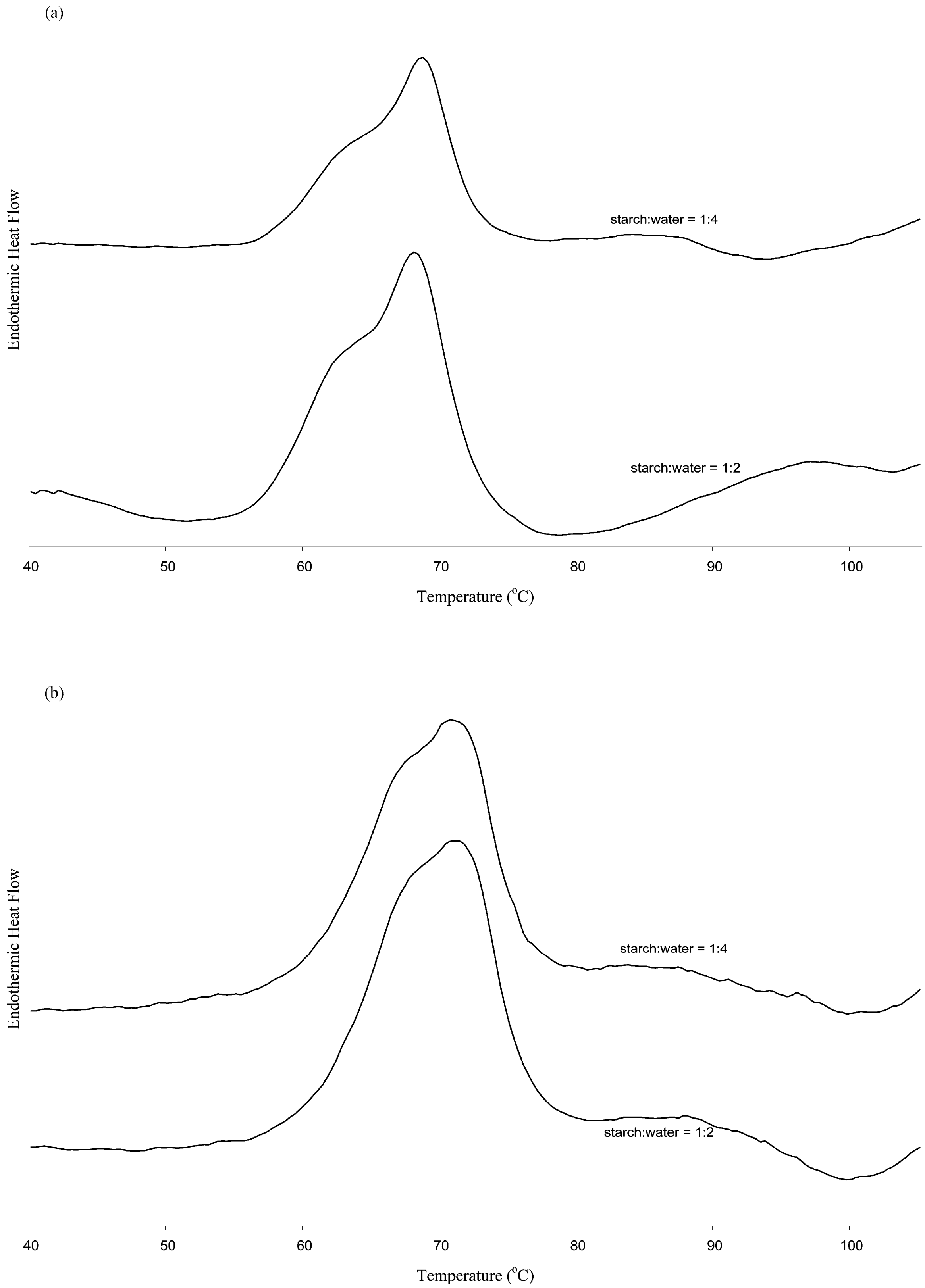


Fig. 3. The effects of water content on DSC parameters and curve shapes of starches from (a) Cuba-23-1-12 and (b) PI-83-9-5.

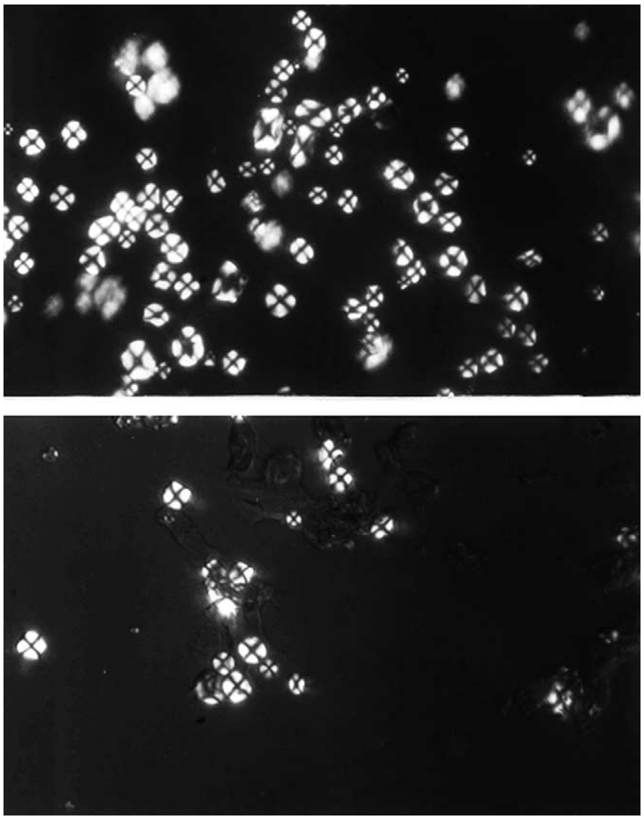


Fig. 4. Polarized-light micrographs of starch from Cuba-23-1-12. (a) Native starch granules. (b) Starch granules after heating to 66 °C, just after reaching the temperature of the first peak shown in Fig. 2(a).

Table 4
Distribution profiles of starch from selected corn lines used in structural analyses

Source of starch (S_{n+1} line[a]-kernel)	Distribution profiles (%)[b]				
	<5 (μm)	5–9 (μm)	9–13 (μm)	13–17 (μm)	≥17 (μm)
Mo17	12.0c[c]	35.2ab	29.0a	18.3b–d	5.5bc
B73	2.7a	30.3ab	33.3ab	21.2b–e	12.5d
Chis-37-5-3	2.3a	30.8ab	48.1de	17.8b–d	1.0ab
Chis-37-11-1	7.4a–c	35.4ab	39.1a–e	16.4a–d	1.7a–c
Cuba-23-1-12	3.3ab	30.7ab	50.6e	15.0a–c	0.4a–c
Cuba-34-1-2	4.1ab	27.2a	39.0ae	22.8c–e	6.9a
Cuba-38-5-5	13.1c	45.5b	34.7a–c	6.6a	0.1a–c
DK-10-1-2	4.2ab	22.2a	38.1a–e	31.1e	4.4a
PI-82-1-1	3.2ab	21.8a	44.0b–e	26.0de	5b
PI-83-2-2	6.6a–c	34.9ab	40.2a–e	16.1a–d	2.2a–c
PI-83-2-5	3.6ab	27.8a	37.8a–d	23.8c–e	7.0c
PI-83-2-10	7.0a–c	32.8ab	40.2a–e	16.7b–d	3.3a–c
PI-83-9-5	4.4ab	37.0ab	46.0c–e	11.4ab	1.2a–c
PI-83-9-8	2.8ab	26.6a	47.2c–e	21.3b–e	2.1a–c
PI-83-9-11	9.3bc	30.7ab	39.1a–e	18.1b–d	2.8a–c

[a] S_{n+1} line means first generation of corn after self-pollination of S_n lines.
[b] Starch granules are divided into different groups according to equivalent diameters.
[c] Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

excluded the possibility that these two transitions were caused by inadequate water content in the DSC run (Donovan & Mapes, 1980). Possibly, these two transitions might correspond to two different starch structures. Furthermore, all starches exhibited a typical A-type diffraction pattern, indicating the starch molecules were packed in a similar fashion within the granule (data not shown).

Based on these initial data, 13 kernels derived from seven exotic corn lines (the eighth exotic line, DK-8, was not further evaluated) were selected for further structural studies of their starch. The exact DSC properties for starch extracted from the specific kernels studied are summarized in Table 2. The data are the average of duplicate DSC analyses of the same starch.

Table 3
Mean granule size and shape parameters of starches from single kernels of selected corn lines used in structural analyses

Source of starch (S_{n+1} line[a]-kernel)	Mean area (μm^2)	Mean diameter (μm)	Mean perimeter (μm)	Mean radial SD (μm)	Mean major axis (μm)
Mo17	88.9b–d[b]	9.8bc–e	41.1b	8.8bc	10.8b
B73	118.8g	11.5g	47.6b	7.4a	12.6c
Chis-37-5-3	91.3b–d	10.4de	43.4c	7.9a	11.3d
Chis-37-11-1	83.1b	9.8b	40.6b	8.3ab	10.6b
Cuba-23-1-12	110.2fg	11.3g	47.6b	8.0a	12.3e
Cuba-34-1-2	62.4a	8.4a	35.3a	8.2ab	9.1a
Cuba-38-5-5	111.9fg	11.4g	48.3d	9.4cd	12.6e
DK-10-1-2	86.3b–d	10.1bc–e	42.7bc	9.5dc	11.1bcd
PI-82-1-1	108.6fg	11.2g	47.6d	9.6de	12.4e
PI-83-2-2	84.8bc	9.8b–d	42.1bc	10.5f	11.6b–d
PI-83-2-5	106.1ef	11.0fg	47.1d	10.5f	12.3e
PI-83-2-10	94.4cd	10.4de	44.2c	10.1d–f	11.6d
PI-83-9-5	82.5	9.8b	41.8b	11.1g	11.1b–d
PI-83-9-8	98.0de	10.7ef	34.8a	10.2ef	12.3e
PI-83-9-11	82.9b	9.6b	41.9bc	11.4g	10.9bc

[a] S_{n+1} line means first generation of corn after self-pollination of S_n lines.
[b] Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

Table 5
Pearson correlation coefficients (r) of DSC properties with granule size and shape distribution

DSC properties	Mean area (μm^2)	Mean radial SD (μm)	Distribution profiles (%)[a]				
			<5 (μm)	5–9 (μm)	9–13 (μm)	13–17 (μm)	>17 (μm)
T_{oG} (°C)	0.04	−0.44*	−0.02	−0.15	−0.62**	0.37	0.73**
T_{pG} (°C)	−0.27	0.05	−0.22	−0.45*	−0.11	0.50*	0.38
ΔH_G (J/g)	−0.22	−0.26	0.03	−0.34	−0.51*	0.48*	0.64**
R_G (°C)	−0.17	0.50*	−0.07	0.00	0.63**	−0.21	−0.66**
PHI	0.18	−0.50*	0.06	−0.05	−0.65**	0.24	0.76**
ΔH_R (J/g)	0.27	0.18	−0.10	−0.40	−0.20	0.46*	0.38
R (%)	0.49*	0.40	−0.11	−0.19	0.13	0.15	−0.05

T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; R_G, range of gelatinization temperature; ΔH_G, enthalpy of gelatinization; ΔH_R, Enthalpy of retrogradation; PHI, peak height index (enthalpy of gelatinization divided by half the range); R, retrogradation. *, **: where p-values for test H_o ($\rho = 0$) versus H_a ($\rho \neq 0$) are smaller than 0.10 and 0.05, respectively.

[a] Starch granules are divided into different groups according to equivalent diameter.

3.2. Polarized microscopy study

The two independent transitions noted during gelatinization of some of the unusual starches were further studied by observing granular birefringence with polarized microscopy (Fig. 4(a) and (b)). After being heated beyond the temperature of the first transition (66 °C) and below the temperature of the second transition (69 °C), some granules were completely gelatinized, whereas others still showed the intact 'Maltese Cross' indicating crystalline structure (Fig. 4(b)). It can be concluded that these two transitions were located in different granules, which differentiates them from those reported in C-type granules characteristic of pea starch (Bogracheva, Morris, Ring, & Hedley, 1998). The two independent gelatinization transitions in C-type starch granules are caused by the coexistence of A and B polymorphs within the same granule. The B polymorphs are arranged centrally with the A polymorphs located peripherally within the granules. During heating in excess salt solution, the polymorphs in the two regions melt independently, giving a double peak in heat capacity; the B polymorphs melting at a lower temperature than the A polymorphs.

3.3. X-ray diffraction pattern of starch

All starches exhibited a typical A-type diffraction pattern, indicating the starch molecules were packed in a similar fashion within the granule. These data are not shown.

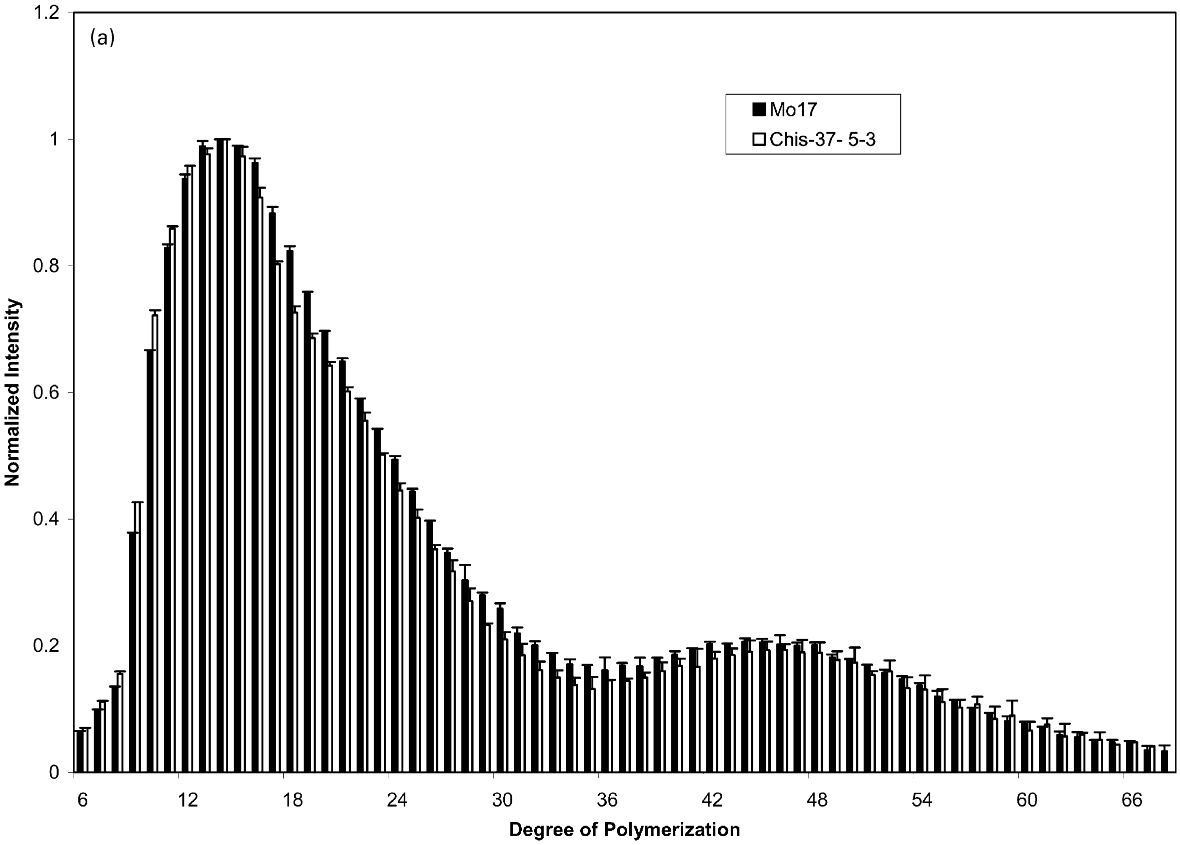
3.4. Granule-size distribution

Significant differences were observed in the mean granule-size parameters among the selected starches (Table 3). Granules of starch from Cuba-34-1-2 had the smallest mean area, equivalent diameter, and major axis, whereas granules of starch from B73 had the greatest values for the same parameters. The mean granule-shape parameters also showed significant differences. Granules from B73 tended to deviate least from a spherical shape (radial SD = 7.4), whereas granules from PI-83-9-11 tended to deviate most from spherical shape (radial SD = 11.6).

Starch granules were divided into five groups according to their equivalent diameters: <5 μm, ≥5 and <9 μm, ≥9 and <13 μm, ≥13 and <17 μm, and ≥17 μm, and reported as a percentage of the total number of granules measured (Table 4). Significant differences were observed in the percentage distribution profiles of some of these selected starches. In general, Chis-37-5-3 had the lowest proportion of granules lower than 5 μm in size (2.3%), whereas Cuba-38-5-5 had the greatest proportion of granules lower than 5 μm in size (13.1%). Cuba-38-5-5 tended to have the lowest proportion of large granules (0.1% of granules ≥17 μm), and B73 had the highest proportion of large granules (12.5% of granules ≥17 μm). Generally, the granule size and shape distributions of all exotic lines studied were different from both B73 and Mo17 starches.

Even though Mo17 and B73 starches had similar DSC properties, granules from these two lines were different in size and shape distributions: granules from B73 were larger and more spherical than granules from Mo17. In addition, B73 starch had a lower percentage distribution of small granules (<5 μm) and greater percentage distribution of larger granules (≥17 μm) than did Mo17. No significant differences were observed in percentage distribution of granules within the ranges of 5–9, 9–13, and 13–17 μm.

Relationships between starch DSC properties and granule size and shape distributions were analyzed by using the Pearson correlation test (Table 5). The DSC parameters of T_{oG}, ΔH_G and PHI were positively correlated with percentage of granules having an equivalent diameter of ≥17 μm ($r = 0.73$, 0.64, and



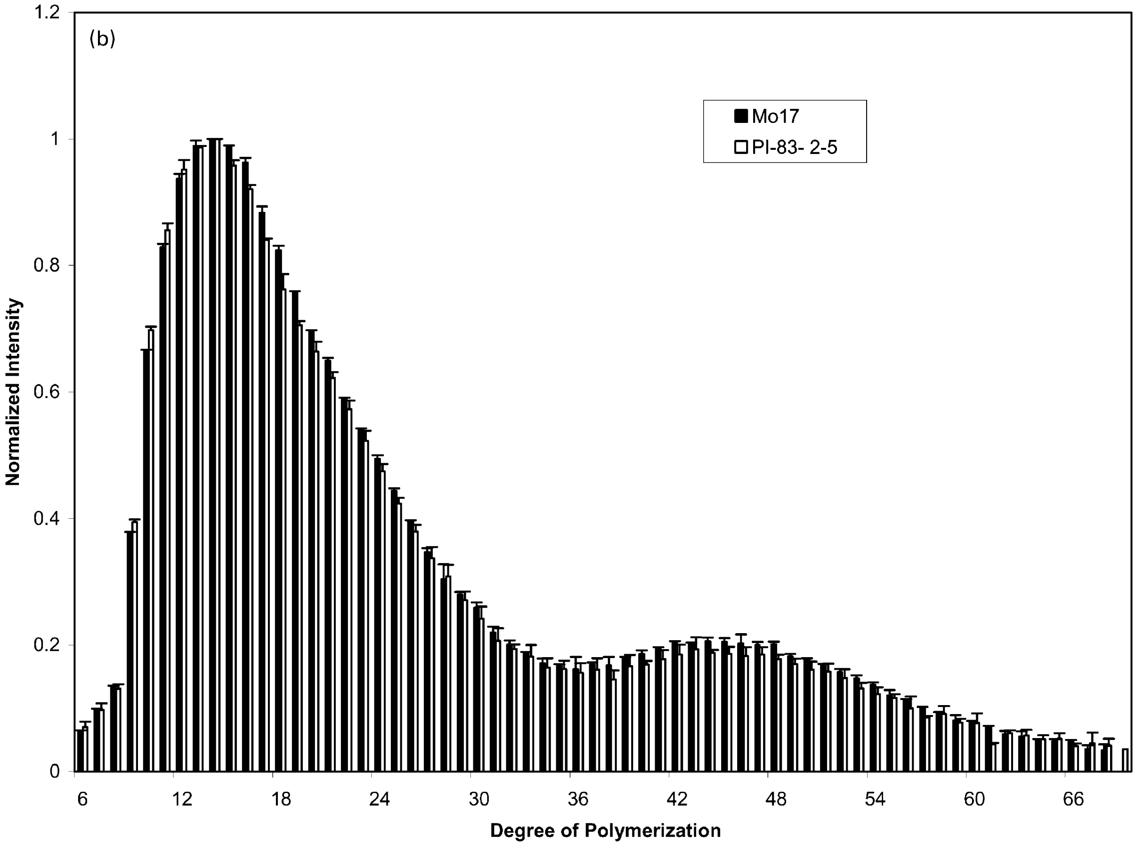


Fig. 5. (a–c) Normalized branch-chain-length distributions of selected exotic starches compared with Mo17 starch determined by using a high-performance anion-exchange chromatography system equipped with an enzyme column reactor and a pulsed amperometric detector. A Carbopac PA100 column composed of immobilized amyloglucosidase was used for the analysis.

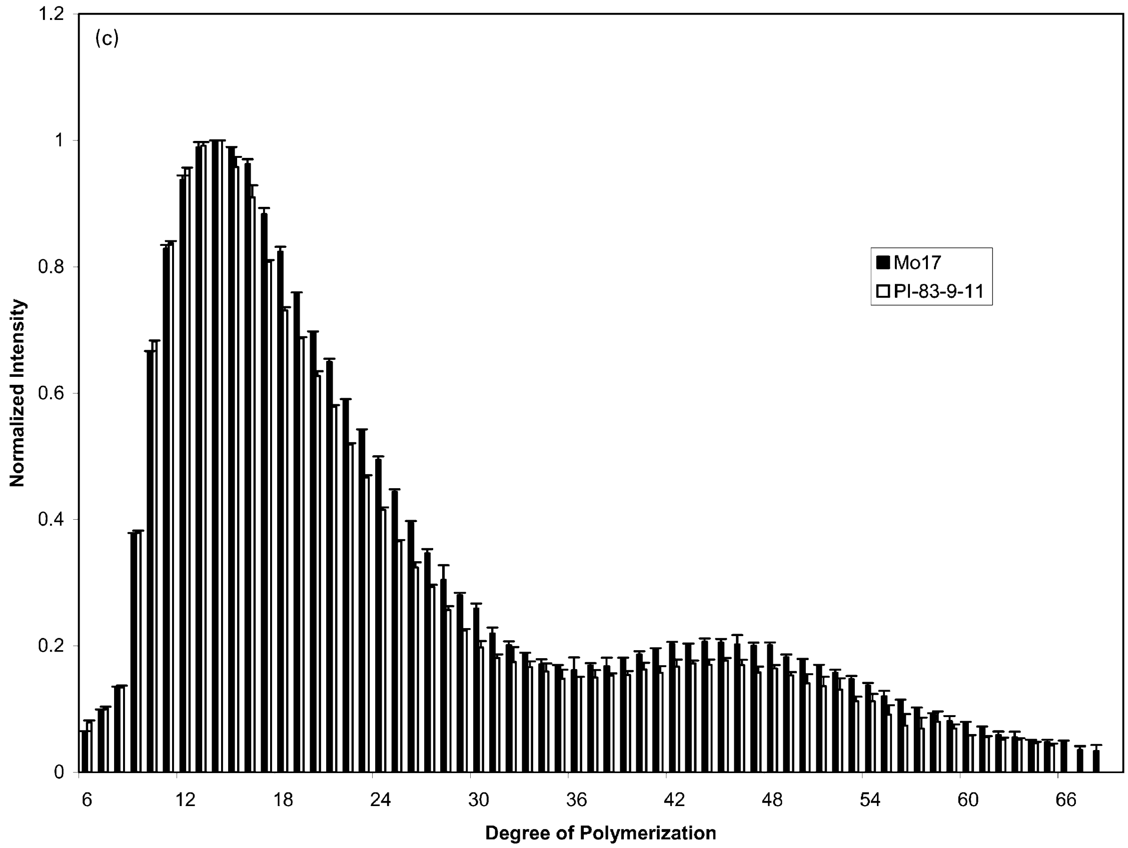


Fig. 5 (*continued*)

0.76, respectively), whereas R_G was negatively correlated with percentage of granules having an equivalent diameter of ≥ 17 μm ($r = -0.66$). Previous studies have noted a negative correlation between T_{oG} and granule-size distribution (Campbell et al., 1996). It is likely that the T_{oG} is influenced by many factors, including that of granule size. For example, small crystals (granules) are more thermodynamically unstable than are large ones (Atkins, 1998), because of the larger surface area, given that both have the same internal structure. Internal structural differences in starch granules also cause size variations in the granule, thus influencing the ultimate granule size. Thus, if only granule size is related to T_{oG}, it might appear that a correlation exists, whereas the correlation is actually a secondary effect of internal structural composition. Another example of differences in composition of large and small granules is the lipid content, which tends to be greater in smaller granules (Banks, Greenwood, & Muir, 1973). No strong correlation was found between DSC properties and mean areas of starch granules.

3.5. Molecular size of amylose

The Sepharose CL-2B gel-permeation chromatograms showed no significant differences in the molecular sizes of amylose among the starches analyzed. The average distribution coefficients (K_0) of amylose for all starches ranged from 0.733 to 0.750 (data not shown).

3.6. Branch-chain-length distribution of amylopectin

Normalized branch-chain-length distributions of the selected starches and of Mo17 and B73 starches were measured by HPAEC-ENZ-PAD (Fig. 5(a)–(c), Table 6). In Fig. 5(a)–(c), three starch types representing slightly different patterns of peak concentrations compared with the control starches are presented. The results are expressed as means ± standard deviation of a minimum of duplicate analyses, and the height of each bar at each degree of polymerization (dp) represents its relative concentration to peak I at dp 13 or 14. In the figures, starches were compared with Mo17 starch. The exotic and B73 corns are Stiff Stalk types, and Mo17 is a non-Stiff Stalk type, but both Mo17 and B73 have branch-chain-length distribution patterns similar to each other. All starches showed a bimodal distribution with the first peak at dp 13–14 and the second peak at dp 42–46. Chains with dp ~ 13–14 were most frequent in the distribution, explaining why all starches displayed an A-type diffraction pattern (Jane et al., 1999). In general, A-type starches contain branches with shorter chain lengths (first peak at dp 12–14, second peak at dp 41–51)

Table 6
Relative branch-chain-length (CL) distributions of whole starch from single kernels of selected corn lines

Source of starch (S_{n+1} line[a]-kernel)	Peak dp[b]		Average CL	% Distribution[c]			
	I	II		dp 6–12	dp 13–24	dp 25–36	dp ≥ 37
Mo17	14	45	25.0	15.64	47.21	15.79	21.36
B73	14	47	25.0	16.34	45.94	16.03	21.09
Chis-37-5-3	14	46	24.5	17.57	46.90	14.35	21.17
Chis-37-11-1	14	45	23.8	17.88	48.66	14.04	19.41
Cuba-23-1-12	13	44	23.2	18.01	50.33	14.02	17.63
Cuba-34-1-2	13	46	23.7	17.81	47.92	16.13	18.15
Cuba-38-5-5	13	45	24.3	17.08	46.12	16.83	19.97
DK-10-1-2	14	44	24.4	16.98	48.41	13.67	20.91
PI-82-1-1	14	46	24.5	16.73	47.78	14.89	20.61
PI-83-2-2	14	46	24.4	17.10	47.85	14.90	20.16
PI-83-2-5	14	43	24.5	16.69	47.12	15.80	20.39
PI-83-2-10	14	46	24.3	16.65	47.83	15.95	19.56
PI-83-9-5	14	45	24.8	16.40	47.65	14.28	21.67
PI-83-9-8	14	45	24.4	16.72	46.70	20.49	24.42
PI-83-9-11	14	45	23.9	17.68	48.45	14.73	19.14

[a] S_{n+1} line means first generation of corn after self-pollination of S_n lines.
[b] dp, degree of polymerization.
[c] Grouping of dp numbers followed that of Hanashiro, Abe, and Hizukuri (1996).

than do B-type starches (first peak at dp 14–16, second peak at dp 48–53). All exotic starches had either lower normalized concentrations of long branch-chain at dp 15–24 or higher normalized concentration of short branch-chain at dp 6–12 (or both these characteristics) than did Mo17 and B73 (Fig. 5(a)–(c)). To classify the starches, the chains were recorded as belonging to one of four fractions: dp 6–12, 13–24, 25–36, or >37, corresponding to A, B1, B2, and B3 or longer chains, respectively, based on Hizukuri's model (1986). All exotic starches had relatively lower average chain lengths (dp 23.2–24.8) than did Mo17 and B73 starches (dp 25), and contained a higher proportion of A chains at dp 6–12 (16.40–18.01%) than did Mo17 (15.64%) and B73 (16.34%) starches (Table 6). Cuba-23-1-12 starch had the shortest average chain length (dp 23.2) and the highest proportion of A chains (dp 6–12) and B1 chains (dp13–24) among all the starches.

3.7. Relationship between gelatinization behavior and granular structure

Gelatinization properties are influenced by the crystalline structure of the starch granule. Results suggest that exotic starches with a shoulder peak or double gelatinization peaks are composed of two different kinds of granules. Even though the starch molecules were packed in an A-type crystalline structure, the granules that gelatinized at a lower temperature may have fewer stable crystallites than those granules that gelatinized at a higher temperature.

Amylopectin is the main component responsible for the crystallinity of starch. Therefore, exotic starches with low T_{oG}, wide R_G, and unsymmetrical gelatinization peaks may have different amylopectin structures than do normal starches. This theory is supported by the fact that amylopectin from exotic starches had different amylopectin branch-chain-length distribution patterns than did normal starch (Fig. 5(a)–(c) and Table 6). According to Hizukuri's model (1986), A chains and B1 chains (those chains that make up the first peak in the distribution profiles of Fig. 5(a)–(c)) are primary participants in the crystalline regions. All starches studied contained a greater proportion of A chains with dp 6–12 and fewer B1 chains with dp 15–24 than did starch from Mo17 and B73. Also, relative intensities of the shoulder at dp 15–24 to peak I in unusual starches were lower than those in Mo17 and B73, which implies that fewer chains in the exotic starches would be long enough to go through the crystalline region, thus resulting in defects in the crystallites. That is to say, crystalline regions in the starches were not as tightly packed as in Mo17 and B73 starch. This observation is corroborated by the DSC data that show lower than normal gelatinization onset temperatures ($T_{oG} \leq 63$ °C) for all exotic starches of interest (Table 2).

4. Conclusions

Exotic corn germplasm is a valuable source for producing starches with useful unique characteristics. The starches from the corn lines identified in this study are of interest because of unusually low T_{oG} and wide R_G. Two independent gelatinization transitions, one corresponding to the melting of a peak at a lower temperature ~66 °C and the other to a peak melting at a higher temperature ~69 °C, located in different granules were found in some starches. All starches exhibited a typical A-type X-ray diffraction pattern. Significant differences were observed in starch-granule size-distributions and shape-distributions of the selected starches. No strong correlation was found between DSC properties with mean areas of starch granules. However, strong correlations were found between DSC properties and proportion of large granules with equivalent diameter ≥ 17 μm. No significant differences were observed in the starch component profiles, as measured by gel-permeation chromatography. The low T_{oG} is consistent with the branch-chain-length pattern of the amylopectin reported here. Starches with a lower T_{oG} had a lower normalized concentration of chains with a dp of 15–24 and/or a greater normalized concentration of chains with a dp of 6–12. Overall starches with a low T_{oG} had a higher relative concentration of branch chains below dp 13 than did normal starch. Work is in progress to genetically ‘fix’ the unusual thermal properties in each line of corn so that succeeding generations exhibit the property, and to better understand the structure–function relationships in these starches.

Acknowledgements

The authors thank the NRI Competitive Grants program/USDA, award number 98355036371, and the Iowa Corn Promotion Board for funding the research.

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