

Effects of Amylopectin Branch Chain Length and Amylose Content on the Gelatinization and Pasting Properties of Starch¹

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ABSTRACT

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Structures and properties of starches isolated from different botanical sources were investigated. Apparent and absolute amylose contents of starches were determined by measuring the iodine affinity of defatted whole starch and of fractionated and purified amylopectin. Branch chain-length distributions of amylopectins were analyzed quantitatively using a high-performance anion-exchange chromatography system equipped with a postcolumn enzyme reactor and a pulsed amperometric detector. Thermal and pasting properties were measured using differential scanning calorimetry and a rapid viscoanalyzer, respectively. Absolute amylose contents of most of the starches studied were lower than their apparent amylose contents. This difference correlated with the number of very long branch chains of amylopectin. Studies of amylopectin structures showed that each starch had a distinct branch chain-length distribution profile. Average

degrees of polymerization (dp) of amylopectin branch chain length ranged from 18.8 for waxy rice to 30.7 for high-amylose maize VII. Compared with X-ray A-type starches, B-type starches had longer chains. A shoulder of dp 18–21 (chain length of 6.3–7.4 nm) was found in many starches; the chain length of 6.3–7.4 nm was in the proximity of the length of the amylopectin crystalline region. Starches with short average amylopectin branch chain lengths (e.g., waxy rice and sweet rice starch), with large proportions of short branch chains (dp 11–16) relative to the shoulder of dp 18–21 (e.g., wheat and barley starch), and with high starch phosphate monoester content (e.g., potato starch) displayed low gelatinization temperatures. Amylose contents and amylopectin branch chain-length distributions predominantly affected the pasting properties of starch.

Starch consists of two polysaccharides, amylose and amylopectin. Amylose has long linear chains of (1→4)-linked α -D-glucopyranose residues, some with a few (>10) branches (Hizukuri et al 1981). Amylopectin has large molecular weight and highly branched structures consisting of much shorter chains of (1→4) α -D-glucose residues. The branch-chains are connected by (1→6)- α -D-glucosidic linkages (Hizukuri 1986). Some starches also contain a third component known as the intermediate component (Lansky et al 1949). Most starches contain 20–30% amylose and 70–80% amylopectin; ratios vary with the botanical source of the starch.

The amylose-to-amylopectin ratio of starch greatly affects the starch functional properties. However, amylose contents of starches reported in literature are mostly apparent amylose (Morrison and Laignelet 1983, Knutson and Grove 1994). Takeda and Hizukuri (1987) have reported that starches isolated from indica rices have more apparent amylose contents than do japonica rices. However, indica rice amylopectins have longer chains than japonica rice amylopectins. Because long chains of amylopectin also can form a helical complex with iodine, the iodine affinity and amylose content of the starch are inflated. The absolute (real) amylose contents of the indica and japonica rice starches are found to be similar. These results show that amylopectin with long branch chains cause overestimation of the amylose content in starch when it is determined by iodine titration or by the blue value method. The study of Kasemsuwan et al (1995) confirms that long branch chains of amylopectin interact with iodine and result in a higher iodine affinity of the starch.

Amylopectin is the major component of most starches, and its fine structure plays a critical role in the characteristics of starch. Studies have revealed that the branch chain-length of amylopectin is related to the starch crystalline structure (Hizukuri et al 1983, Hizukuri 1985, Gidley and Bulpin 1987, Hanashiro et al 1996). The branch chain length also affects the gelatinization, retrogradation (Miles et al 1985; Gudmundsson and Eliasson 1990; Kalichevsky et al 1990; Jane et al 1992; Shi and Seib 1992, 1995; Yuan

et al 1993; Lu et al 1997), and pasting properties of starch (Jane et al 1992, Jane and Chen 1992, Wang et al 1993). The relationship between the amylopectin branch chain length and the functional properties of starch isolated from different botanical sources was difficult to establish because different methods were used by different investigators. Detailed information about the relationship between the structure and functional property of starch is needed to provide direction for genetic modifications.

In this study, the apparent and absolute amylose contents of starch were determined by measuring the iodine affinity of defatted whole starch and of the fractionated pure amylopectin. The branch chain-length distribution of amylopectin was quantitatively determined by using a high-performance anion-exchange chromatography system equipped with a postcolumn enzyme reactor and a pulsed amperometric detector (HPAEC-PAD-ENZ) (Wong and Jane 1997). Thermal properties (e.g., gelatinization and retrogradation) and pasting properties of the starch were studied by using differential scanning calorimetry and a rapid viscoanalyzer, respectively. Results obtained from these studies were used to establish correlation between chemical structures and functional properties of starches.

MATERIALS AND METHODS

Materials

Chinese taro, mung bean, waxy rice, sweet rice, green leaf canna, lotus root, water chestnut, and cattail millet starches were isolated in the laboratory as reported by Lim et al (1994). Waxy amaranth I was isolated by the alkaline method (Myers and Fox 1994). Waxy amaranth II and normal glacier barley starch were isolated by a combination of mild alkaline and protease treatments (Radosavljevic et al 1998). Green banana starch was provided by A. R. Bonilla, University of Costa Rica. Waxy maize, amylose-extender (*ae*) waxy and dull (*du*) waxy maize starches were gifts of Cerestar, USA (Hammond, IN); potato and wheat starches were purchased from Sigma Chemical Co. (St. Louis, MO); rice starch was from Matheson Coleman & Bel Inc. (Cincinnati, OH); large granule barley starch was a gift of Oyakoab Co. (Finland); normal glacier barley was a gift of Walter Newman (Montana State University); and tapioca, normal maize, and high-amylose maize (V) and (VII) starches were gifts of National Starch and Chemical Co. (Bridgewater, NJ). Isoamylase (EC 3.2.1.68) from *Pseudomonas amyloferamosa* was purchased from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan), and amyloglucosidase (EC

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3.2.1.3) from *Rhizopus* mold (9032-80-0) was purchased from Sigma and was used without further purification.

Methods

Amylose and a mixture of amylopectin and intermediate components were separated following a modified Schoch method (Jane and Chen 1992). The mixture of amylopectin and intermediate components was purified by recrystallizing five times to remove amylose residues. The purity of amylopectin was examined by gel-permeation chromatography monitored by the total carbohydrate and blue value (Jane and Chen 1992).

Iodine affinities of defatted starch and of isolated amylopectin were measured by using an automatic potentiometric titrator (702 SM Tirino, Metrohm, Herisau, Switzerland) following the method previously reported (Kasemsuwan et al 1995). Apparent amylose contents were calculated by dividing the iodine affinity of defatted starch by 20% (Takeda and Hizukuri 1987). Absolute amylose contents were calculated by the method of Kasemsuwan et al (1995). Each analysis was replicated a minimum of three times.

Amylopectin was debranched by isoamylase following the procedure of Jane and Chen (1992). The amylopectin 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 the method reported by Wong and Jane (1997). A DX-300 gradient chromatography system, a CarboPac PA100 (4 × 250 mm) column, a CarboPac PA 100 guard column (3 × 25 mm), and an enzyme column (2 × 23 mm) immobilized with amyloglucosidase were employed. The pulsed potentials and durations were E1 = 0.05 V (t1 = 480 msec), E2 = 0.60 V (t2 = 120 msec), and E3 = -0.60 V (t3 = 60 msec). The eluents A and B were 100 mM sodium hydroxide and 100 mM sodium hydroxide in 300 mM sodium nitrate solution, respectively. The gradient of eluent B was 1% at 0 min, 5% at 39 min, 8% at 50 min, 30% at 170 min, and 45% at 220 min. The eluent gradient was operated at a 0.5 mL/min flow rate. The results were obtained from at least two replicates of each amylopectin sample.

Starch X-ray patterns were obtained with copper, nickel foil-filtered, K_{α} radiation by using a diffractometer (D-500, Siemens, Madison, WI). The diffractometer was operated at 27 mA and 50 kV. The scanning region of the diffraction angle (2θ) was from 4° with

a 0.05° step size and a count time of 2 sec. Starch samples were equilibrated in a 100% rh chamber at 25°C for 24 hr before measurement.

Gelatinization and retrogradation properties of starches were analyzed by using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) equipped with an intracooling II system. Aluminum pans (Perkin-Elmer) were used for the analyses. Starch samples (≈ 2 mg each, dsb) were weighed in sample pans, mixed with distilled water (≈ 6 mg), and sealed. The heating rate was 10°C/min over the temperature range of 25–100°C. Stainless steel pans (Perkin-Elmer) were used for high-amylase maize V and VII starches at a heating range of 25–150°C. Indium and zinc were used as the reference standards. Enthalpy change (ΔH), gelatinization onset temperature (T_o), peak temperature (T_p), and gelatinization ranges were computed automatically. The data were averages of a minimum of three replicates of each starch sample. The retrogradation study was performed using the same method with the same sample used for gelatinization study after storage at 4°C for seven days.

Starch pasting properties were analyzed by using a Rapid ViscoAnalyser (RVA) (Newport Scientific, Sydney, Australia). Each starch suspension (8%, w/w, dsb; 28 g of total weight) was equilibrated at 50°C for 1 min, heated at a rate of 6°C/min to 95°C, maintained at that temperature for 5 min, and then cooled to 50°C at a rate of 6°C/min. A constant rotating speed of the paddle (160 rpm) was used. Because of the limited amount of sample, 5% (w/w, dsb) of green leaf canna starch suspension was used, and it was compared with potato and normal maize starches at the same concentration level.

RESULTS AND DISCUSSION

Absolute Amylose Content

Long branch chains of amylopectin, like amylose, bind iodine to form a single helical complex during potentiometric titration, develop a blue color, and, consequently, inflate the iodine affinity and the apparent amylose content of the starch. Because of this, we examined both the apparent amylose content and the absolute amylose content of each starch, and the results are shown in Table I. Most normal starches, except Chinese taro, had lower absolute amylose contents than their apparent amylose contents. Iodine affin-

TABLE I
Iodine Affinities and Amylose Contents of Starches

Source ^a	Iodine Affinity ^b		Amylose Content (%) ^c		
	Starch	Amylopectin	Apparent (A) ^d	Absolute (B) ^e	A – B
A-type starch					
Normal maize	5.88 ± 0.14	1.78 ± 0.03	29.4	22.5	6.9
Rice	5.00 ± 0.02	1.11 ± 0.00	25.0	20.5	4.5
Wheat	5.75 ± 0.15	0.8 ± 0.2	28.8	25.8	3.0
Barley	5.1 ± 0.3	0.50 ± 0.04	25.5	23.6	1.9
Cattail millet	3.97 ± 0.06	1.08 ± 0.03	19.8	15.3	4.5
Mung bean	7.58 ± 0.06	2.07 ± 0.03	37.9	30.7	7.2
Chinese taro	2.75 ± 0.06	0.00 ± 0.00	13.8	13.8	0.0
Tapioca	4.7 ± 0.2	1.39 ± 0.07	23.5	17.8	5.7
B-type starch					
Amylomaize V	10.4 ± 0.1	6.79 ± 0.04	52.0	27.3	24.7
Amylomaize VII	13.6 ± 0.2	9.3 ± 0.1	68.0	40.2	27.8
Potato	7.20 ± 0.01	4.6 ± 0.1	36.0	16.9	19.1
Green leaf canna	8.64 ± 0.00	5.31 ± 0.03	43.2	22.7	20.5
C-type starch					
Water chestnut	5.79 ± 0.19	3.08 ± 0.02	29.0	16.0	13.0

^a Determined by X-ray diffraction.

^b Averaged from at least three replicates ± standard deviation of each sample, except for defatted green leaf canna starch, of which the iodine affinity was averaged from two replicates.

^c Iodine affinity for pure amylose was assigned as 20% (Takeda and Hizukuri 1987).

^d Calculated as: $C = 100 \times IA_S / 0.20$ where C is the percentage of apparent amylose content and IA_S is the iodine affinity of the whole defatted starch.

^e Calculated as: $C = (IA_S - IA_{AP+IC}) / \{0.20 - (IA_{AP+IC} / 100)\}$ where C is the percentage of absolute amylose content, IA_S is the iodine affinity of whole defatted starch, and IA_{AP+IC} is the iodine affinity of the amylopectin and the intermediate component mixture.

ities of waxy starches, which contained no amylose, are shown in Table II. Among the waxy starches, *ae* waxy maize starch displayed an apparent amylose content of 34.5%, despite the starch containing no amylose. Starches displaying a B-type X-ray pattern, which had larger proportions of long amylopectin branch chains than the A- and C-type starches (Table III), gave greater over-estimations of the amylose content (Tables I and II). The results of HPAEC-ENZ-PAD chromatography (Table III) showed that amylopectin without detectable very long branch chains (dp > 73) (Chinese taro amylopectin, waxy rice, and waxy maize starches), displayed no detectable iodine affinity (Tables I and II). These results indicated a positive correlation between the iodine affinity of amylopectin and the presence of very long branch chains of the amylopectin instead of long average-branch-chain lengths.

Branch Chain-Length Distribution of Amylopectin

Normalized HPAEC-ENZ-PAD chromatograms of branch chain-length distributions of amylopectins isolated from starches that display A-, B-, and C-type X-ray patterns are shown in Figs. 1–3, respectively, and the computed results are summarized in Table III. In general, A-type starches had peaks at shorter chain-lengths

(first peak at dp 12–14, second peak at dp 41–51) (Fig. 1) than B-type starches (first peak at dp 14–16, second peak at dp 48–53) (Fig. 2). A-type starches also had larger proportions (15.6–27.4%) of short chains (dp 6–12) and smaller proportions (6.6–26.7%) of long chains (dp > 37) than B-type starches, 8.5–12.3% and 26.1–29.5% for the short and long chains, respectively (Table III). The C-type starches had substantial amounts of both short and long branch-chains (Fig. 3). Although some trends of amylopectin branch chain-length distributions were observed for starches with the same crystalline type, each starch had its own chain-length distribution profile (Figs. 1–3). For example, all the cereal starches, and many other starches, displayed very few short chains of dp 6 and a gradual increase in chains of dp 7–9. Some tuber, root, and legume starches such as Chinese taro, lotus root, water chestnut, and mung bean starch displayed a higher population of dp 7 than dp 6 and 8. Potato amylopectin had the lowest population of dp 8. These results were in agreement with those reported by Hanashiro et al (1996). The HPAEC-ENZ-PAD chromatograms yielded more detailed results of the branch chain-length distribution of amylopectins, especially for the long branch chains.

Most starch samples analyzed displayed a shoulder at dp 18–21, which has been reported by Hanashiro et al (1996). The relative intensity of the shoulder differed with starches. Among the starches studied, wheat, barley, *du* waxy maize, amaranth, and tapioca had the most obvious shoulders. Amylopectins of *ae* waxy maize and high-amylose maize V and VII also displayed shoulders; however, the relative intensities of the shoulders were close to the peak height, much higher than other starches (Fig. 2). Physical distances of starch chains with dp 18–21 are calculated to be 6.3–7.4 nm, using 0.35 nm for the distance of each glucose anhydrous unit. The range of the distance, 6.3–7.4 nm, was in the proximity of the length of amylopectin crystalline region, 6.65 nm for wheat, reported by Cameron and Donald (1992). The results suggested that dp 18–21 represented the full length of the crystalline region, and the ratio of the intensities of peak 1 and the shoulder indicated the proportion of short chains in the crystallites that result in defects. Starches that did not show a shoulder in the chromatogram include normal maize, normal rice, cattail millet, Chinese taro, potato, green-leaf canna, and green banana.

TABLE II
Iodine Affinities and Apparent Amylose Contents of Waxy Starches

Source ^a	Iodine Affinity ^b	Apparent Amylose Content ^c (%)
A-type starch		
Waxy maize	0.00 ± 0.00	0.0
<i>du</i> Waxy maize	0.42 ± 0.03	2.1
Waxy rice	0.00 ± 0.00	0.0
Sweet rice	0.41 ± 0.03	2.1
Waxy amaranth	0.68 ± 0.03	3.4
B-type starch		
<i>ae</i> Waxy maize	6.89 ± 0.19	34.5

^a Determined by X-ray diffraction.

^b Iodine affinities were averaged from at least three replicates ± standard deviation. Iodine affinity for pure amylose was assigned as 20% (Takeda and Hizukuri 1987).

^c Calculated as: $C = 100 \times IA_s / 0.20$ where C is the percentage of apparent amylose content and IA_s is the iodine affinity of the whole defatted starch.

TABLE III
Branch Chain-Length (CL) Distributions of Amylopectins^a

Source ^b	Peak dp		Average CL	% Distribution					Highest Detectable dp
	I	II		dp 6–9	dp 6–12	dp 13–24	dp 25–36	dp ≥ 37	
A-type starch									
Normal maize	13	48	24.4	3.85	17.9	47.9	14.9	19.3	80
Waxy maize	14	48	23.5	6.94	17.0	49.4	17.1	16.5	73
<i>du</i> Waxy maize	14	51	23.1	1.21	16.7	51.9	17.4	14.0	80
Normal rice	12	46	22.7	4.10	19.0	52.2	12.3	16.5	80
Waxy rice	12	41	18.8	8.57	27.4	53.4	12.6	6.6	66
Sweet rice	12	45	21.6	7.59	23.5	48.7	13.7	14.0	78
Wheat	12	41	22.7	5.18	19.0	41.7	16.2	13.0	77
Barley	12	43	22.1	4.92	20.8	48.9	17.7	12.6	75
Waxy amaranth	12	43	21.8	6.31	25.1	47.7	12.5	14.7	80
Cattail millet	13	45	21.5	4.21	20.2	53.8	12.7	13.3	74
Mung bean	13	48	24.8	2.45	15.6	47.6	18.3	18.5	74
Chinese taro	13	45	23.4	5.99	18.8	48.7	14.8	17.7	71
Tapioca	12	49	27.6	4.68	17.3	40.4	15.6	26.7	79
B-type starch									
<i>ae</i> Waxy maize	16	53	29.5	2.29	10.4	43.5	18.1	28.0	84
Amylomaize V	16	48	28.9	1.90	9.7	43.9	20.3	26.1	86
Amylomaize VII	16	48	30.7	1.81	8.5	40.7	21.3	29.5	86
Potato	14	52	29.4	3.53	12.3	43.3	15.5	28.9	85
Green leaf canna	15	52	28.9	3.41	11.7	45.3	16.2	26.8	85
C-type starch									
Lotus root	13	52	25.4	4.57	16.4	47.2	15.4	21.0	83
Water chestnut	13	50	26.7	5.86	17.8	43.7	15.3	23.2	80
Green banana	13	48	26.4	5.25	16.8	46.3	12.9	24.0	79

^a Grouping of degree of polymerization (dp) numbers followed that of Hanashiro et al (1996).

^b Determined by X-ray diffraction.

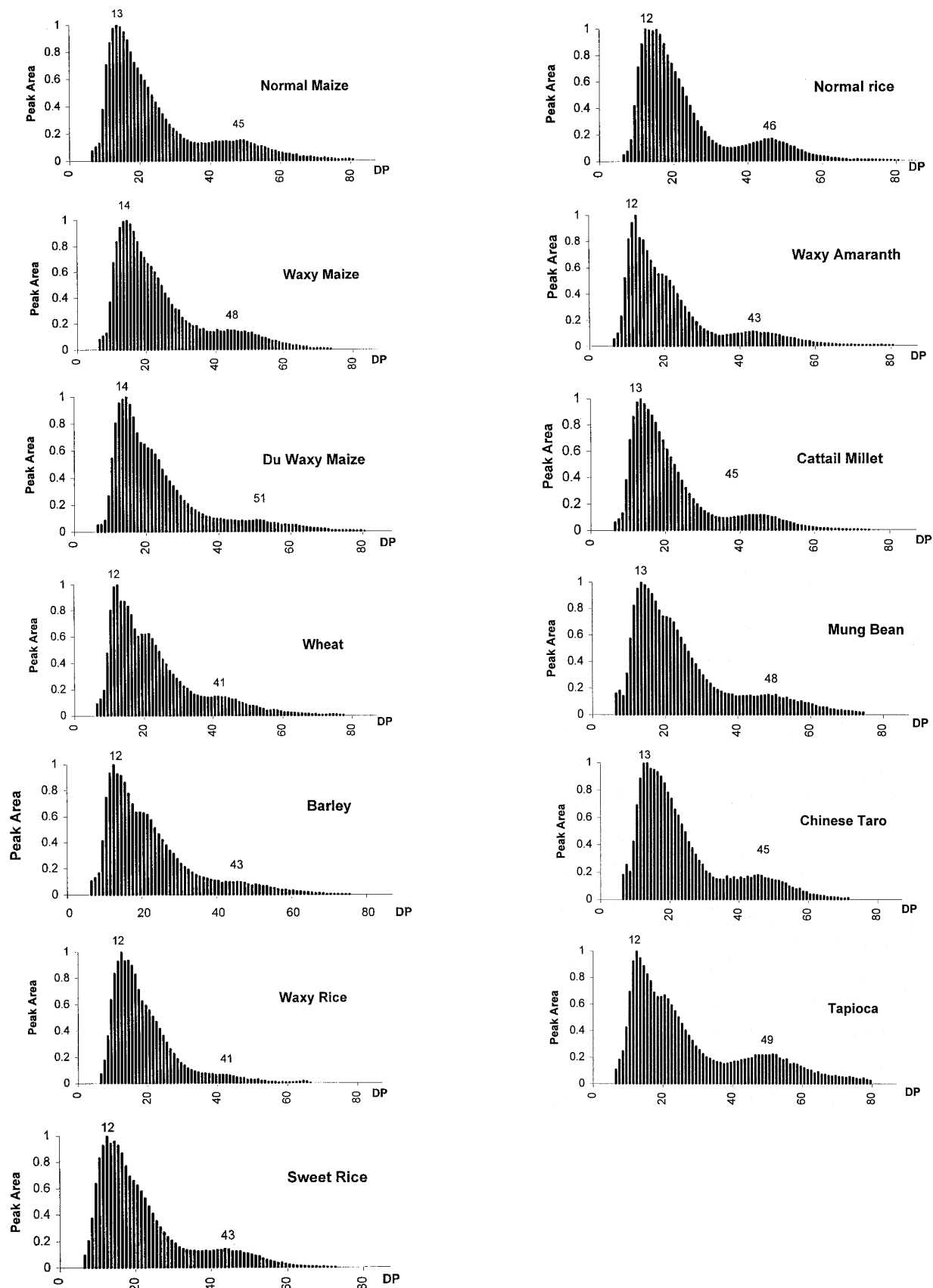


Fig. 1. Amylopectin branch chain-length distributions of A-type starches determined by using a high-performance anion-exchange chromatography system equipped with an enzyme column reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD). A CarboPac PA100 column and an immobilized amyloglucosidase column were used for the analysis.

Starch Gelatinization

Gelatinization properties of starches measured by differential scanning calorimetry (DSC) are summarized in Table IV. Among the starch samples, waxy rice, wheat, barley, and potato displayed the lowest onset gelatinization temperatures. Waxy rice starch had the shortest average branch chain length (dp 18.8), the shortest second peak chain length (dp 41), the largest proportion of short chains (dp 6–12, 27.4%), very few long chains, and a shoulder at dp 18. Studies with rice starches (Asaoka et al 1985), taro starch (Jane et al 1992), and waxy maize starches (Shi and Seib 1992, 1995, Yuan et al 1993) have shown that those with shorter long-B chains display lower gelatinization temperatures. Wheat and barley starches, with average chain lengths of dp 22.1–22.7 and relatively short second peak chain lengths (dp 41 and 43, respectively), displayed obvious shoulders at dp 18–21. The relatively high ratio of the intensities of peak 1 to the shoulder suggested a defective crystalline structure, which could contribute to the very low gelatinization temperature. Potato starch had long branches with an average branch chain length of dp 29.4, but this starch contained the highest concentration of phosphate monoester derivatives. The phosphate monoester derivatives, in addition to having a B-polymorph, contributed to the low gelatinization temperature. With the same chain length, starch of the B-polymorph display a lower gelatinization temperature than that of the A-polymorph (Whittan et al 1990). This characteristic could be attributed to the presence of 36 molecules of water in a unit cell of the B-polymorph (Sarko and Wu 1978, Imberty et al 1991). Starch of the A-polymorph is more densely packed with ≈ 8 water molecules in a unit cell (Sarko and Wu 1978). Starches that displayed high gelatinization temperatures, such as Chinese taro, green banana, cattail millet, normal rice, *ae* waxy, and high-amylose maize V and VII, in general, displayed no shoulder or a shoulder of very high relative intensity (e.g., *ae* waxy and high-amylose maize starches). Gelatinization temperature ranges of starches varied from 6.6°C (barley) to 58.8°C (high-amylose maize VII) (Table IV).

Caution is needed for the study of starch gelatinization properties because many growing and processing conditions can change the gelatinization temperature of starch. Different methods used for starch isolation were observed to affect the gelatinization temperature of starch. For example, waxy amaranth starch isolated by

a strong-alkaline method (Myers and Fox 1994) displayed a significantly higher gelatinization temperature (70.4°C) than the 66.7°C for starch isolated from the same sample by a mild alkaline and enzymatic method (Radosavljevic et al 1998).

Waxy starches are known to display larger gelatinization enthalpy changes, reflecting a higher percentage crystallinity of amylopectin. Starches with longer branch chain length, such as high-amylose maize V and VII, *ae* waxy maize, and potato, also displayed larger enthalpy changes, indicating larger amounts of energy were needed to gelatinize crystallites of longer chain lengths.

Starch Retrogradation

DSC data of starch retrogradation are summarized in Table V. Onset thermal transition temperatures of dissociating retrograded starches after storage at 4°C for seven days ranged between 37.3 and 46.6°C and were lower than the onset gelatinization temperature of native starches (56.9–71.5°C). The percentage retrogradation (%R), calculated by $\Delta H_{\text{retrograded starch}} / \Delta H_{\text{gelatinization}}$, ranged from 4.3% for sweet rice starch to 80.8% for high-amylose maize V.

Starches that displayed the lowest retrogradation rates were waxy rice, sweet rice, and waxy amaranth; however, not all waxy starches displayed low retrogradation rates. Waxy maize starch displayed a retrogradation rate similar to that of normal maize starch (–47%); *du* waxy maize and *ae* waxy maize starches both displayed among the highest retrogradation rates (71.2 and 61.6%, respectively). Starch of *ae* waxy maize with very long branch chain length (average dp 29.5) and the longest second peak chain length (dp 53) was expected to have a high retrogradation rate. Interestingly, *du* waxy maize starch had a relatively short average chain length (dp 23.1) compared with waxy maize starch (dp 23.5) but had a very long second peak chain length (dp 51) and the lowest proportion (1.21%) of short chains of dp 6–9. ^{31}P -NMR studies have shown that *du* waxy maize starch contains phospholipids, which is rare among waxy starches (Lim et al 1994). Retrogradation rates of starches were inversely correlated with the proportion of short chains of dp 6–9 as proposed by Shi and Seib (1992), but no correlation was found with the molar proportion of unit chains with dp 14–24. Results suggested that amylopectin molecules with branched structures can be equally effective for crystalline structure formation, depending on the structure of branch chains.

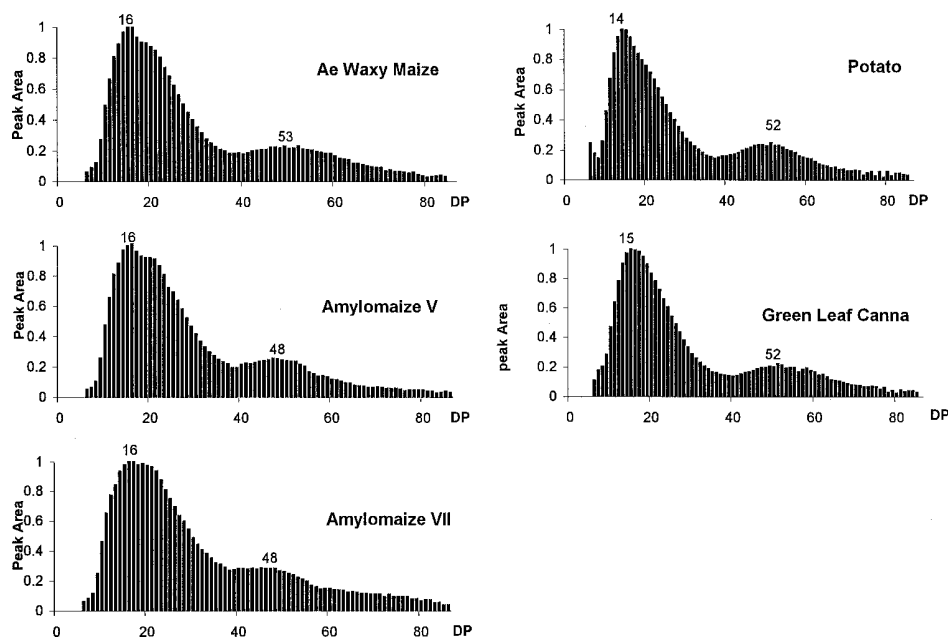


Fig. 2. Amylopectin branch chain-length distributions of B-type starches determined by using a high-performance anion-exchange chromatography system equipped with an enzyme column reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD). A Carbowac PA100 column and an immobilized amyloglucosidase column were used for the analysis.

Results of this study showed that cereal starches, in general, retrograded more rapidly than the tuber and root starches. This could be attributed to the presence of phosphate monoesters in the tuber and root starches (Lim et al 1994), which retard retrogradation. In contrast, the presence of lipids and phospholipids in cereal starches and some waxy cereal starches (e.g., *du* waxy maize starch) (Lim et al 1994), which restrict the starch granule swelling and dispersion, expedite retrogradation.

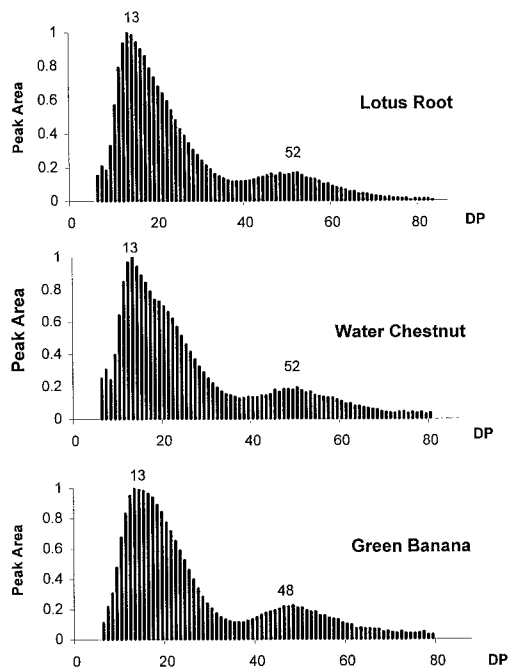


Fig. 3. Amylopectin branch chain-length distributions of C-type starches determined by using a high-performance anion-exchange chromatography system equipped with an enzyme column reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD). A Carbowac PA100 column and an immobilized amyloglucosidase column were used for the analysis.

Pasting Properties

Pasting viscosity profiles of starches analyzed by using RVA are shown in Fig. 4, and results are summarized in Table VI. Pasting properties of starch are affected by amylose and lipid contents and by branch chain-length distribution of amylopectin. Amylopectin contributes to swelling of starch granules and pasting, whereas amylose and lipids inhibit the swelling (Tester and Morrison 1990). Furthermore, the amylopectin chain-length and amylose molecular size produce synergistic effects on the viscosity of starch pastes (Jane and Chen 1992). Thus, the effects of the structural features on the pasting properties of starches are rather complex.

The RVA results showed that the pasting temperatures of all starches were higher than the onset gelatinization temperatures (T_o) determined by DSC. The differences ranged between $\approx 3^\circ\text{C}$ of waxy amaranth and tapioca starches and $>30^\circ\text{C}$ of wheat and barley starches. In general, waxy cereal starches (except *du* waxy maize and *ae* waxy maize) had lower pasting temperatures, higher peak viscosity, and lower set-back viscosity than the normal starch counterparts (Fig. 4a and b and Table VI). Waxy starches consisted mainly of amylopectin and, thus, the swelling of granules was not restricted by amylose-lipid complex. With no amylose present in the waxy starches examined in this study, set-back viscosity, which reflects gel network formation involving amylose, was generally low. Starches of *du* waxy and *ae* waxy maize (Fig. 4a), however, had different pasting profiles from the aforementioned waxy starches. Both starches had relatively high pasting temperatures (75.7 and 83.2°C , respectively), lower peak viscosity (109 and 162 RVU, respectively) and smaller breakdown in viscosity during the holding period at 95°C . It is plausible that the very long branch-chains of amylopectin mimic amylose to form helical complexes with lipids and intertwine with other branch chains to hold the integrity of starch granules during heating and shearing.

The amylose-lipid complexes in normal starches, such as normal maize (Fig. 4a), normal rice (Fig. 4b), wheat, and barley (Fig. 4c), caused an increase in pasting temperature and increased resistance to shear-thinning of starch pastes. This effect has been more pronounced in wheat and barley starches because both have high concentrations of phospholipids (0.053% phosphorus in the wheat [Lim et al 1994] and 0.052% in the barley [Song and Jane, *in press*]).

TABLE IV
Thermal Properties of Starch Gelatinization Determined by Differential Scanning Calorimetry^a

Type	T_o ($^\circ\text{C}$)	T_p ($^\circ\text{C}$)	T_c ($^\circ\text{C}$)	Range ($^\circ\text{C}$)	ΔH (J/g)
A-type starch					
Normal maize	64.1 ± 0.2	69.4 ± 0.1	74.9 ± 0.6	10.8	12.3 ± 0.0
Waxy maize	64.2 ± 0.2	69.2 ± 0.0	74.6 ± 0.4	10.4	15.4 ± 0.0
<i>du</i> Waxy maize	66.1 ± 0.5	74.2 ± 0.4	80.5 ± 0.2	14.4	15.6 ± 0.2
Normal rice	70.3 ± 0.2	76.2 ± 0.0	80.2 ± 0.0	9.9	13.2 ± 0.6
Waxy rice	56.9 ± 0.3	63.2 ± 0.3	70.3 ± 0.7	13.4	15.4 ± 0.2
Sweet rice	58.6 ± 0.2	64.7 ± 0.0	71.4 ± 0.5	12.8	13.4 ± 0.6
Wheat	57.1 ± 0.3	61.6 ± 0.2	66.2 ± 0.3	9.1	10.7 ± 0.2
Barley	56.3 ± 0.0	59.5 ± 0.0	62.9 ± 0.1	6.6	10.0 ± 0.3
Waxy amaranth	66.7 ± 0.2	70.2 ± 0.2	75.2 ± 0.4	8.5	16.3 ± 0.2
Cattail millet	67.1 ± 0.0	71.7 ± 0.0	75.6 ± 0.0	8.5	14.4 ± 0.3
Mung bean	60.0 ± 0.4	65.3 ± 0.4	71.5 ± 0.4	11.5	11.4 ± 0.5
Chinese taro	67.3 ± 0.1	72.9 ± 0.1	79.8 ± 0.2	12.5	15.0 ± 0.5
Tapioca	64.3 ± 0.1	68.3 ± 0.2	74.4 ± 0.1	10.1	14.7 ± 0.7
B-type starch					
<i>ae</i> Waxy maize	71.5 ± 0.2	81.0 ± 1.7	97.2 ± 0.8	25.7	22.0 ± 0.3
Amylomaize V	71.0 ± 0.4	81.3 ± 0.4	112.6 ± 1.2	41.6	19.5 ± 1.5
Amylomaize VII	70.6 ± 0.3	nd ^b	129.4 ± 2.0	58.8	16.2 ± 0.8
Potato	58.2 ± 0.1	62.6 ± 0.1	67.7 ± 0.1	9.5	15.8 ± 1.2
Green leaf canna	59.3 ± 0.3	65.4 ± 0.4	80.3 ± 0.3	21.0	15.5 ± 0.4
C-type starch					
Lotus root	60.6 ± 0.0	66.2 ± 0.0	71.1 ± 0.2	10.5	13.5 ± 0.1
Green banana	68.6 ± 0.2	72.0 ± 0.2	76.1 ± 0.4	7.5	17.2 ± 0.1
Water chestnut	58.7 ± 0.5	70.1 ± 0.1	82.8 ± 0.2	24.1	13.6 ± 0.5

^a Onset temperature (T_o), peak temperature (T_p), completion temperature (T_c), and enthalpy change (ΔH) of starch gelatinization. Range of gelatinization is $T_c - T_o$. Values are averages of at least three replicates of each sample.

^b Not detectable.

Therefore, wheat and barley starches had the highest pasting temperatures and very low peak viscosity (Table VI). Because of very high gelatinization temperatures (>100°C), high-amylose maize V and VII starches did not completely gelatinize under the RVA cooking conditions.

Both starches have very high amylose contents (Table I) that did not contribute to swelling, and thus displayed very low

viscosity as shown in Fig. 4a. Cattail millet starch had the lowest absolute amylose content (15.3 %) among normal cereal starches. This feature might explain its high peak viscosity (201 RVU) and low resistance to shear-thinning (hot paste viscosity, 80 RVU). In addition to the effects of amylopectin, amylose, and lipids, the study also showed that different methods used for starch isolation affected the pasting properties. Examples were waxy amaranth I

TABLE V
Thermal Properties of Starch Retrogradation Determined by Differential Scanning Calorimetry^a

Type	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	%R
A-type starch					
Normal maize	39.0 ± 0.3	50.1 ± 0.6	59.4 ± 0.1	5.8 ± 0.5	47.6
Waxy maize	40.2 ± 0.5	51.3 ± 0.0	60.2 ± 0.2	7.3 ± 0.2	47.0
<i>du</i> Waxy maize	37.3 ± 0.1	49.4 ± 0.4	60.4 ± 0.2	11.1 ± 0.2	71.2
Normal rice	40.3 ± 0.4	51.0 ± 0.3	60.4 ± 0.2	5.3 ± 0.2	40.5
Waxy rice	43.2 ± 2.1	50.6 ± 0.1	55.2 ± 1.1	0.8 ± 0.5	5.0
Sweet rice	39.8 ± 0.7	50.5 ± 0.0	54.9 ± 0.5	0.6 ± 0.0	4.3
Wheat	38.6 ± 0.3	47.7 ± 0.9	55.7 ± 0.2	3.6 ± 0.1	33.7
Barley	39.9 ± 0.8	48.7 ± 0.1	56.5 ± 0.2	3.3 ± 0.5	32.5
Waxy amaranth	42.8 ± 0.4	50.7 ± 0.0	55.0 ± 0.4	0.8 ± 0.2	5.2
Cattail millet	40.5 ± 0.7	50.5 ± 0.0	60.0 ± 0.5	7.8 ± 0.0	53.8
Mung bean	39.0 ± 0.7	50.3 ± 0.5	62.3 ± 1.2	6.7 ± 0.8	58.9
Chinese taro	40.8 ± 0.7	51.2 ± 0.2	57.9 ± 0.2	4.8 ± 0.2	32.0
Tapioca	42.1 ± 0.4	51.2 ± 0.5	58.8 ± 0.1	3.7 ± 0.0	25.3
B-type starch					
<i>ae</i> Waxy maize	40.1 ± 1.1	63.0 ± 1.9	87.3 ± 0.4	13.6 ± 0.5	61.6
Amylomaize V	44.1 ± 0.8	nd ^b	120.5 ± 2.1	15.8 ± 1.2	80.8
Amylomaize VII	46.6 ± 0.5	nd	115.4 ± 1.8	9.9 ± 0.5	61.0
Potato	42.5 ± 0.4	55.7 ± 0.6	66.9 ± 1.0	7.5 ± 0.3	43.4
Green leaf canna	41.3 ± 0.3	57.6 ± 0.1	74.0 ± 1.6	7.0 ± 1.2	45.2
C-type starch					
Lotus root	41.0 ± 0.3	51.5 ± 0.0	62.0 ± 0.0	5.8 ± 0.1	43.2
Green banana	40.7 ± 0.2	52.5 ± 0.0	64.4 ± 0.1	8.2 ± 0.1	47.7
Water chestnut	40.1 ± 0.2	51.3 ± 0.0	63.3 ± 0.2	6.5 ± 0.3	47.9

^a Onset temperature (T_o), peak temperature (T_p), completion temperature (T_c), and enthalpy change (ΔH) of dissociating retrograded starch. %R is percentage of retrogradation. Values are averages of at least three replicates of each sample.

^b Not detectable.

TABLE VI
Pasting Properties of Starches Measured by Rapid ViscoAnalyser

Source ^a	Pasting Temperature (°C)	Viscosity (RVU) ^b			
		Peak	Hot Paste	Final	Set-Back
Normal maize	82.0	152	95	169	74
Waxy maize	69.5	205	84	100	16
<i>du</i> Waxy maize	75.7	109	77	99	22
<i>ae</i> Waxy maize	83.2	162	150	190	40
Waxy rice	64.1	205	84	100	16
Sweet rice	64.6	219	100	128	28
Normal rice	79.9	113	96	160	64
Wheat	88.6	104	75	154	79
Barley I ^c	87.8	58	15	54	39
Barley II ^c	91.2	88	58	116	58
Waxy amaranth I ^d	75.0	60	29	35	6
Waxy amaranth II ^d	70.2	125	75	86	11
Cattail millet	74.2	201	80	208	128
Chinese taro	73.1	171	88	161	73
Tapioca	67.6	173	61	107	46
Lotus root	67.4	307	84	138	54
Potato	63.5	702	165	231	66
Mung bean	73.8	186	161	363	202
Green banana	74.0	250	194	272	78
Water chestnut	74.3	61	16	27	11
Pastes ^e					
Normal maize	90.5	28	18	25	17
Potato	64.4	234	94	114	30
Green leaf canna	71.1	183	181	294	113

^a Mixtures consisted of 8% (w/w, dsb) starch in water unless otherwise stated.

^b Measured in Rapid ViscoAnalyser units.

^c Barley I and barley II were commercial large-granule starch and laboratory-isolated starch, respectively.

^d Waxy amaranth I and II were isolated by the alkaline method (Myers and Fox 1994) and the diluted alkaline and protease method (Radosavljevic et al 1998), respectively.

^e Pastes consisted of 5% starch (w/w, dsb).

and waxy amaranth II, isolated by a strong alkaline method (Myers and Fox 1994) and by a low alkaline-protease method (Radosavljevic et al 1998), respectively. Pasting profiles of the two starches (Fig. 4c) showed that the amaranth I starch, isolated by the strong alkaline method, displayed a substantially lower

viscosity and higher pasting temperature, indicating damaged starch.

Among tuber starches, potato starch had an extraordinarily high peak viscosity (702 RVU, Fig. 4d) as a result of the high phosphate monoester content (0.089%, dry basis) (Lim et al 1994; McPherson

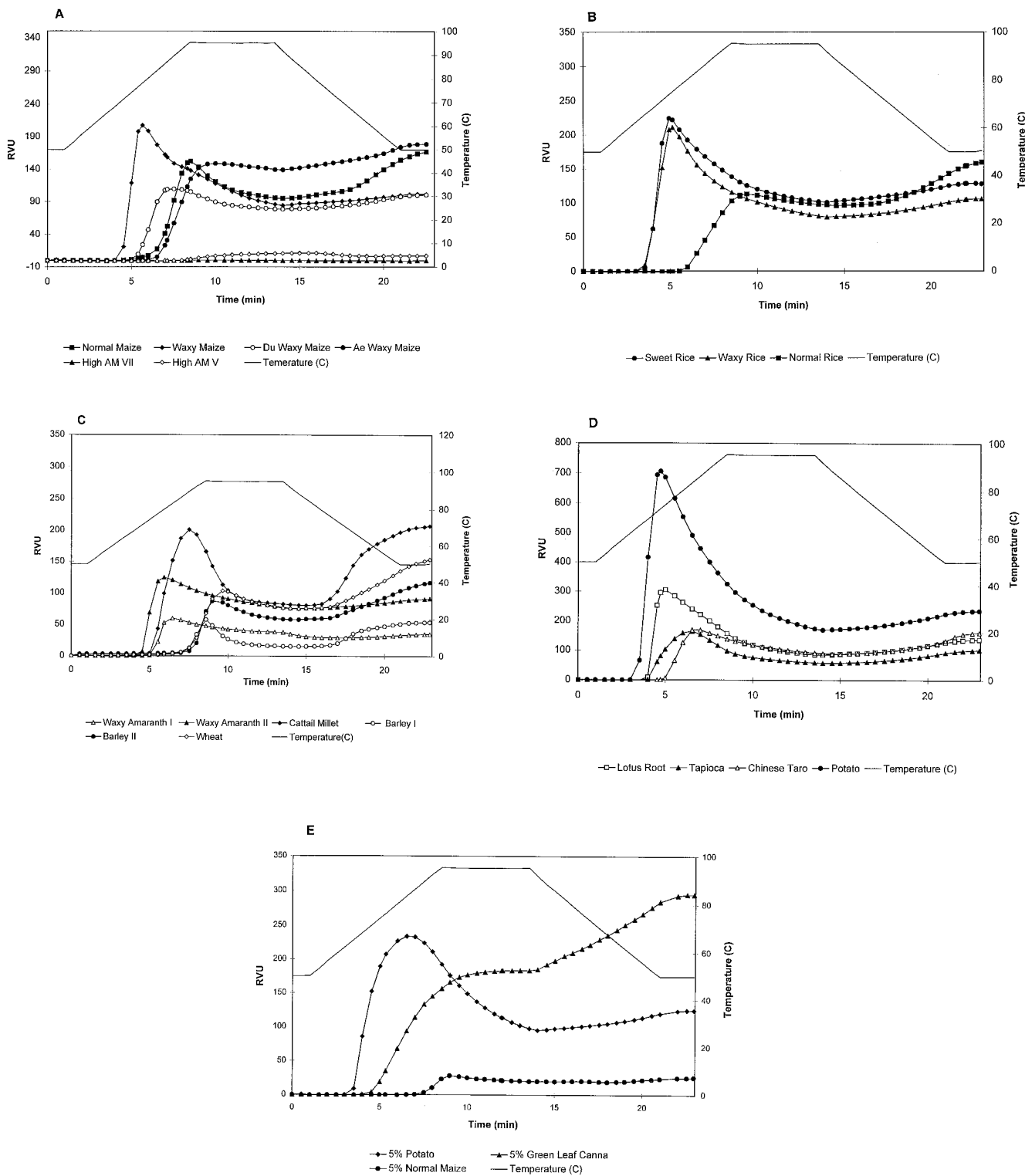


Fig. 4. Pasting profiles of starches (w/w, dsb) measured by rapid viscoanalysis. **A,** Maize mutants at 8% starch. **B,** Rice mutants at 8% starch. **C,** Other cereal starches at 8% starch. Waxy amaranth I and II starches were prepared by an alkaline method (Myers and Fox 1994) and the diluted alkaline and protease method (Radosavljevic et al 1998), respectively. Barley I and II starches were commercial large granule starch and laboratory-isolated glacier barley starch, respectively. **D,** Root and tuber starches at 8% starch. **E,** Potato, green leaf canna, and normal maize at 5% starch.

and Jane 1999) and long branch chains. Compared with normal cereal starches, tuber and root starches had lower pasting temperatures, lower resistance to shear-thinning, and lower set-back viscosities. These properties were attributed to the absence of lipids and phospholipids in the tuber and root starches (Lim et al 1994). Among the tuber and root starches shown in Fig. 4d, Chinese taro starch had the highest pasting temperature and the least shear thinning. These features may relate to its small starch granules (1–4 μm , diameter) (Jane et al 1994). Green leaf canna starch exhibited a very high peak viscosity (183 RVU at 5% concentration) compared with normal maize starch (28 RVU) and potato starch (234 RVU) at the same concentration (Fig. 4e). The starch paste of green leaf canna did not show a breakdown in viscosity during the holding period at 95°C and had a much higher set-back viscosity (113 RVU) than potato starch (30 RVU) and normal maize starch (16 RVU). This unique pasting profile of green leaf canna starch could be attributed to the presence of a high amylose content and high proportions of very long amylopectin branch chains. Mung bean starch, consisting of a high absolute-amylose content (30.7%) also displayed a very high set-back viscosity (202 RVU) (Table VI).

CONCLUSIONS

The studies showed that amylopectin branch chain lengths and distributions determined starch gelatinization temperature, enthalpy change, and pasting properties. Starch gelatinization temperature increased with increasing branch chain-length. Those starches displaying a substantial shoulder at dp 18–21 in the branch chain-length distribution, in general, had lower gelatinization temperatures. Increasing amylose contents, along with lipids and phospholipids, significantly increased starch pasting temperature, decreased peak viscosity and shear thinning, and increased set-back viscosity. The very long branch-chains of amylopectin (dp > 73), like amylose, could bind iodine and inflate the iodine affinity. The very long branch-chains, like amylose, also affected starch-pasting properties. Starches isolated by different methods displayed different physical properties.

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