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Characterization of barley starches of waxy, normal, and high amylose varieties ☆

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Abstract

Four varieties of barley starches, W.B. Merlin, glacier, high amylose glacier, and high amylose hull-less glacier, were isolated from barley seeds. Apparent and absolute amylose contents, molecular size distributions of amylose and amylopectin, amylopectin branch-chain-length distributions, and Naegeli dextrin structures of the starches were analyzed. W.B. Merlin amylopectin had the longest detectable chain length of DP 67, whereas glacier, high amylose glacier and high amylose hull-less glacier amylopectins had the longest detectable chain length of DP 82, 79, and 78, respectively. All the four starches displayed a substantially reduced proportion of chains at DP 18–21. Amylopectins of high amylose varieties did not show significantly larger proportions of long chains than that of normal and waxy barley starch. Onset gelatinization temperatures of all four barley starches ranged from 55.0 to 56.5°C. Absolute amylose contents of W.B. Merlin, glacier, high amylose glacier, and high amylose hull-less glacier were 9.1, 29.5, 44.7, and 43.4%, respectively; phospholipid contents were 0.36, 0.78, 0.79, and 0.97%, respectively.

Keywords: Barley starches; Amylopectins; Phospholipid

1. Introduction

Barley is one of the major sources of cereal starch. Barley grains consist of up to 65% starch (MacGregor & Fincher, 1993). It has been reported that barley starches from different genotypes vary in chemical compositions and properties (Bathgate & Palmer, 1972; Oscarsson, Parkkonen, Autio & Aman, 1997; Schulman, Tomooka, Suzuki, Myllarinen & Hizukuri, 1995; Tester, 1997; Vasanthan & Bhatty, 1996). The analyses of amylose contents and amylopectin chain-length profiles of normal, high amylose, and waxy barley have been done by using gel permeation chromatography (GPC) on Sepharose CL-6B column and by using ^{1}H-NMR spectroscopy after enzyme debranching (Salomonsson & Sundberg, 1994). The results showed that the average amylopectin chain length of high amylose barley starch was five glucose units longer than that of normal and waxy barley starches. Great differences in physicochemical properties of barley starches were reported among different varieties by Vasanthan and Bhatty (1996). Twelve varieties of waxy barley and six normal barley starches were studied for swelling and gelatinization (Morrison & Tester, 1992; Morrison, Tester, Snape, Law & Gidley, 1993; Tester & Morrison, 1990). The results showed that lipid-complexed amylose and free amylose affected the gelatinization properties of starch granules. Properties and chemical structures of Nupana and Titan barley starches have been studied and reported by DeHass and Goering (1972). Their results show that Nupana starch, which displays a higher viscosity, has larger molecules of amylose and amylopectin than Titan starch. This result indicates that starch structure and properties differ between the two barley varieties. In contrast to maize starch counterparts, high amylose glacier and high amylose hull-less glacier barley starches have gelatinization temperatures similar to glacier (normal) and W.B. Merlin (waxy) starches. Relationships between the chemical structures and the thermal properties of these starches were of great interest. In this study, we analyzed the apparent and absolute amylose contents, phosphorus contents, branch-chain-length distributions, and branch structures of the amylopectins by examining the structures of Naegeli dextrins. The structures of the starches were compared with their thermal and pasting properties.

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2. Materials and methods

2.1. Materials

Four varieties of barley starches, W.B. Merlin, glacier, high amylose glacier, and high amylose hull-less glacier, were provided by Dr. Newman, Montana State University. Protease from *Aspergillus sojae* was purchased from Sigma Chemical Co. (St. Louis, MO). Isoamylase from *Pseudomonas amyloderamosa* was purchased from Hayashibara Biochemical Laborataries, Inc. (Okayama, Japan). Other chemicals, all reagent grade, were used without purification.

2.2. Isolation of starch

Whole barley kernels were cracked slightly by a blender before steeping in 0.05% NaOH solution (pH 12) for 24 h under propeller stirring. The steeping solution was discarded and the kernels were washed with water, then the sample was blended for 5 min by using an Osterizer blender at full speed (Radosavljevic, Jane & Johnson, 1998). Protease (Type XIV, from *Aspergillus*, 0.35 units/mg solid) was added (5 mg/g grain) to the sample at pH 7.5. The slurry was mixed in the blender for 1 min at full speed and then incubated in a shaker water bath at 37°C and 50 rpm for 2 h. After incubation, the slurry was filtered through a nylon screen (30 μm) with additional distilled water for washing the fiber fraction. The fiber fraction was blended with additional water until no more starch was released. The solids obtained by centrifugation were purified by additional protease XIV treatment (5 mg/g of starch in 30 ml of incubation mixture) and four times by the toluene shaking procedure (McDonald & Stark, 1988). The clean white layer was washed with water and methanol before drying in a convection oven at 40°C for 48 h.

2.3. Fractionation of amylose and amylopectin

Fractionation of amylose and amylopectin was carried out by following the general procedure of Schoch (1942) and Jane and Chen (1992). The procedure consisted of heating and stirring a starch dispersion (1.33%, w/v in water) in a water bath at 100°C until starch gelatinized. Starch solutions were filtered to remove insoluble residues. The pH of the solution was adjusted to 5.9–6.3 with a phosphate buffer and then autoclaved at 121°C for 3 h. The flask containing the starch solution was stirred in a boiling water bath for 2 h to disperse starch molecules. *n*-Butyl alcohol was added (20%, v/v), and the solution was stirred at 100°C for 1 h under reflux. The sealed flask with mixture was placed in a Dewar flask that was filled with boiling water. The Dewar flask was then sealed and allowed to cool down to room temperature over a period of 24–36 h. Amylose–butyl-alcohol complex crystals formed and precipitated during cooling. The crude amylose–butyl-alcohol complex was separated by centrifuging ($8700 \times g$, 30 min). The amylopectin remaining in the supernatant was concentrated by using a rotary evaporator and then treated twice more with *n*-butyl-alcohol to remove amylose residues. The solution was further concentrated and precipitated with excess methyl alcohol.

2.4. Scanning electron microscopy

Scanning electron micrographs (SEM) of the starches were taken with a scanning electron microscope (JEOL JSM-35, Tokyo, Japan) at Bessey Microscopy Facility, Iowa State University. Starch samples were suspended in ethanol and placed on silver tape attached to a brass disk. The specimens were coated with gold–palladium (60:40). Micrographs of each starch sample were taken at 1500 × magnification (Jane, Kasemsuwan, Leas, Zobel & Robyt, 1994).

2.5. X-ray diffraction pattern

Starch samples were equilibrated in a 100% relative humidity chamber for 24 h at room temperature. X-ray patterns of the starches were obtained with copper, nickel foil-filtered, Kα radiation using a diffractometer (D-500, Siemens, Madison, WI). The diffractometer was operated at 27 mA and 50 kV. The scanning region of the two-theta angle (2θ) was from 4 to 40° with a 0.05° step size and a count time of 2 s.

2.6. Gel-permeation chromatography

Starch molecular-weight distribution profiles were determined by using a gel-permeation chromatography (GPC) column (2.6 cm i.d. × 90 cm) packed with Sepharose CL-2B gel (Pharmacia Inc., Piscataway, NJ), following the method of Jane and Chen (1992). Starch (0.5 g) was moistened with 5 ml of water, and dimethyl sulfoxide (DMSO) (45 ml) was added. The suspension was mechanically stirred while heating in a boiling water bath for 1 h and then stirred for 24 h at 25°C to prepare a starch solution (1%). An aliquot (2 ml) of the starch solution was mixed with absolute ethyl alcohol (8 ml) to precipitate the starch, followed by centrifugation. The precipitated starch was redissolved in boiling water (10 ml) and stirred for 30 min, and the solution was filtered to remove the insoluble residues. The supernatant (5 ml) containing starch (15 mg) and glucose (0.75 mg, as a marker) then was injected into the column. The column was run in the ascending mode. A solution made of distilled water containing 25 mM NaCl and 1 mM NaOH was used as an eluent at a flow rate of 30 ml/h. Fractions of 4.8 ml per cup were collected and analyzed for total carbohydrate (anthrone–sulfuric acid method) and blue value (iodine staining) by using Auto-analyzer II (Technicon Instruments Corp., Elmsford, NY) at 630 and 640 nm, respectively (Jane & Chen, 1992).

2.7. Apparent and absolute amylose contents

Apparent amylose contents were determined by measuring

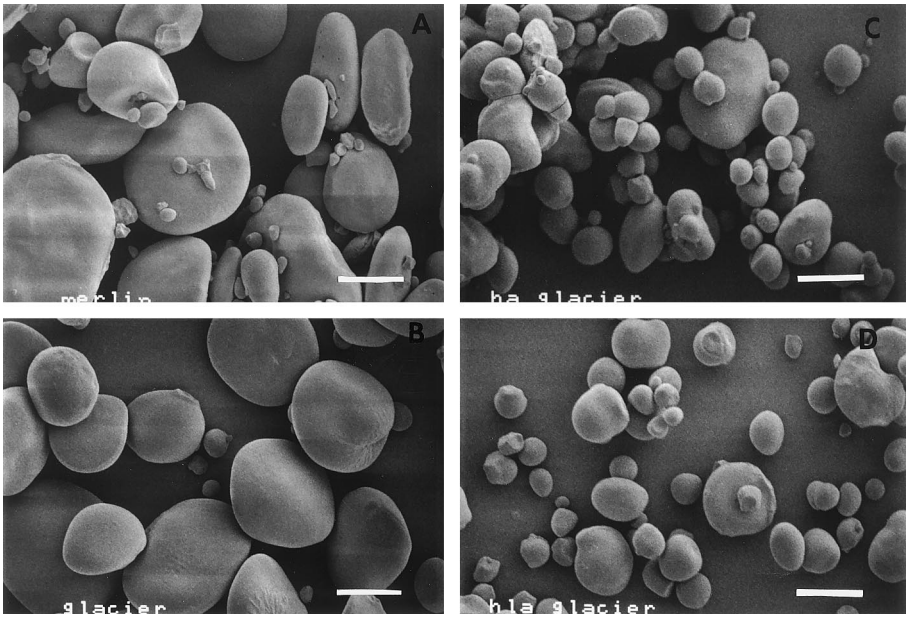

Fig. 1. Scanning electron micrographs of barley starches: (A) W.B. Merlin; (B) glacier; (C) high amylose glacier; (D) high amylose Hull-less glacier. Bar = 10 μm.

iodine affinities of defatted starches using a potentiometric autotitritor (702 SM Titrino, Brinkmann Instrument, Westbury, NY). The analysis was based on the method of Schoch (1964) and Kasemsuwan, Jane, Schnable and Robertson (1995). Iodine affinities of the starch samples (I_{starch}) were analyzed in duplicate. Iodine affinity ($I_{amylose}$) of 20% for amylose was used for calculation (Takeda & Hizukuri, 1987). The iodine affinity of amylopectin ($I_{amylopectin}$) was determined by using pure amylopectin following the same method of Schoch (1964). "Absolute" amylose contents were calculated by using the formula $(I_{starch} - I_{amylopectin})/(I_{amylose} - I_{amylopectin}) \times 100\%$ (Takeda, Takeda & Hizukuri, 1983).

2.8. Phosphorus analysis

The phosphorus contents of barley starches were determined by a colorimetric chemical method (Smith & Caruso, 1964). ^{31}P-NMR was used to characterize and quantify the phosphorus structures and contents in starch, using nicotinamide adenine dinucleotide (NAD) as an internal reference standard (Kasemsuwan & Jane, 1996). The total phosphorus content of each starch was calculated as the sum of all the phosphorus contents. Phosphorus contents of the starches were calculated by multiplying phosphorus contents in the form of phospholipids with 16.16 (Morrison & Tester, 1992).

2.9. Amylopectin branch chain-length distribution

Amylopectin (10 mg) in 90% DMSO solution (1 ml) was precipitated with methyl alcohol (4 ml). The precipitate was dissolved in distilled water (9 ml) by heating and stirring in a boiling water bath for 1 h. The solution was cooled to room temperature (25°C), and 1 ml of acetate buffer (0.1 M, pH 3.5) and isoamylase (150–300 units) were added. The mixture was incubated for 48 h in a shaker bath at 40°C and 120 strokes/min. The sample solution was then adjusted to pH 6 by adding sodium hydroxide solution dropwise and heated in a boiling water bath for 15 min to inactivate the enzyme. Sodium azide was added to the sample solution (0.02%) to prevent microorganisms from growing.

The branch chain-length distributions of amylopectins were analyzed by using high-performance anion-exchange chromatography equipped with an amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) (Dionex, Sunnyvale, CA) following the procedures reported by Wong and Jane (1997). Each sample was analyzed in duplicate.

2.10. Preparation and structure analysis of Naegeli dextrins

A rapid method of preparation of Naegeli dextrin was used (Umeiki & Kainuma, 1981). The starch sample (20 g, dsb) was suspended in a 15% (v/v) H_2SO_4 solution (400 ml) and held at 38°C in an incubator. Starch suspensions were gently shaken daily by hand. Samples were taken after 12 days. The supernatant was siphoned off. An aliquot of the supernatant was analyzed for total carbohydrate content to calculate the starch hydrolyzed. The starch residues were washed with ethanol and then dried at 38°C. The chain-length distributions of Naegeli dextrins before and after isoamylase debranching were analyzed by using the HPAEC-ENZ-PAD system as described earlier.

2.11. Thermal properties

Gelatinization and retrogradation properties of starches were analyzed using a differential scanning calorimeter

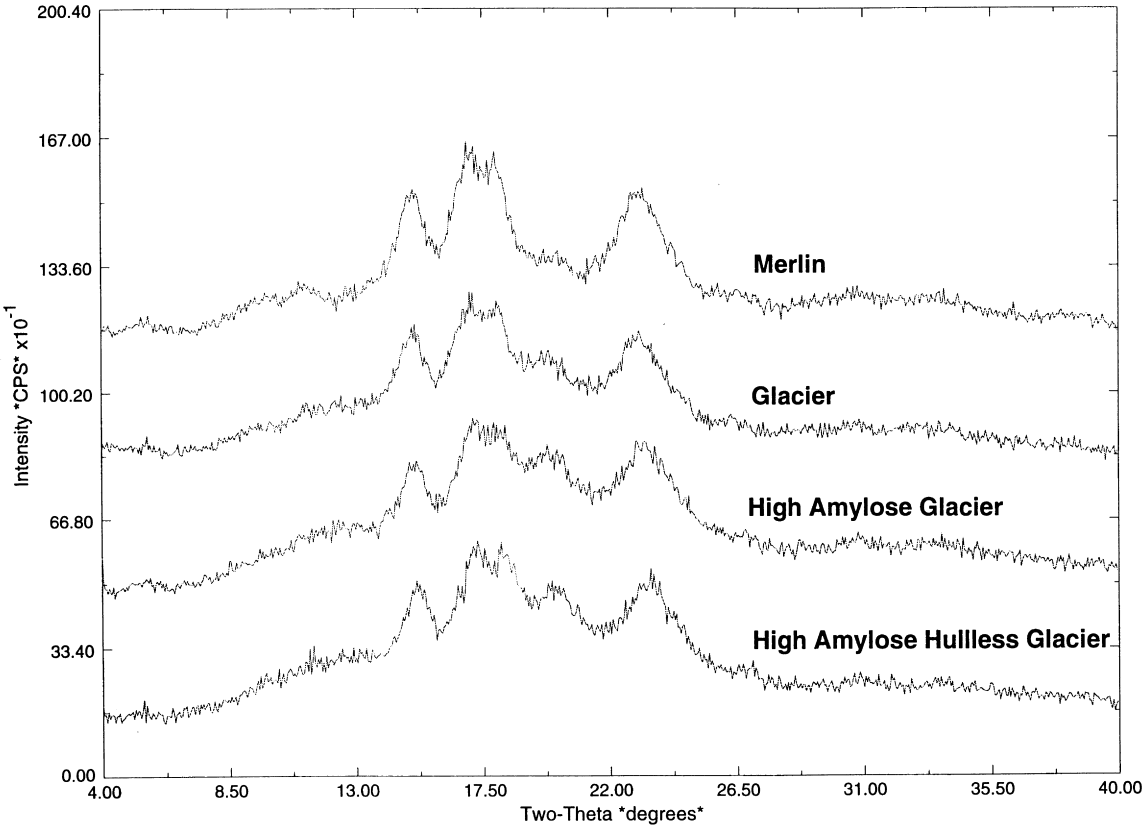

Fig. 2. X-ray patterns of barley starches: (a) W.B. Merlin; (b) glacier; (c) high amylose glacier; (d) high amylose hull-less glacier.

(DSC-7, Perkin–Elmer, Norwalk, CT) equipped with an intracooling II system. Aluminum pans (Perkin–Elmer) were used for the analysis. Starch samples (about 2 mg each, dsb) were weighed in the sample pans, mixed with distilled water (about 6 mg), and sealed. The heating rate was at 10°C per min over the temperature range of 25–120°C. Indium and zinc were used as the reference standards. Enthalpy change (ΔH), gelatinization onset temperature (T_0), peak temperature (T_p), and gelatinization ranges were measured and calculated by using Pyris software (Perkin–Elmer, Norwalk, CT). The data were averages of a minimum of three replicates of each starch sample. The retrogradation study was performed following the same method using the same gelatinized starch samples that had been stored at 4°C for 7 days.

2.12. Pasting properties

Pasting properties of the starches were determined by using a Rapid Visco-Analyzer (RVA) (Newport Scientific, Sydney, Australia). Each starch suspension (8%, dsb, w/w; 28 g total weight) was equilibrated at 50°C for 1 min and then heated at a rate of 6°C/min to 95°C and then maintained at that temperature for 5 min. The sample was then cooled to 50°C at a rate of 6°C/min. A rotating speed of the paddle (160 rpm) was used except the paddle speed was 960 rpm at the first 10 s.

3. Results and discussion

3.1. Granule morphology and crystalline structure

Barley starch granules showed bimodal size distributions (Fig. 1). Large (A) granules had diameters of 15–25 μm, and small (B) granules had 2–5 μm. The results were in agreements with those reported by Jane et al. (1994) and MacGregor and Fincher (1993). Scanning electron micrographs showed that W.B. Merlin and glacier starches had greater proportions of A granules, whereas high amylose and high amylose hull-less glacier starches had A granules with smaller diameters than did glacier and W.B. Merlin starches. W.B. Merlin starch had a greater proportion of B granules than did glacier starch. All barley starches displayed an A-type X-ray diffraction pattern (Fig. 2). High-amylose glacier, high-amylose hull-less glacier, and glacier barley starches also displayed a minor peak at $2\theta = 20°$ which reflected the presence of amylose–lipid complex (Zobel, 1988).

3.2. Starch molecular size distribution and amylose and phosphorus contents

Gel permeation chromatograms of the four varieties of barley starches are shown in Fig. 3. The first peak in the profile corresponded to amylopectin. The second peak

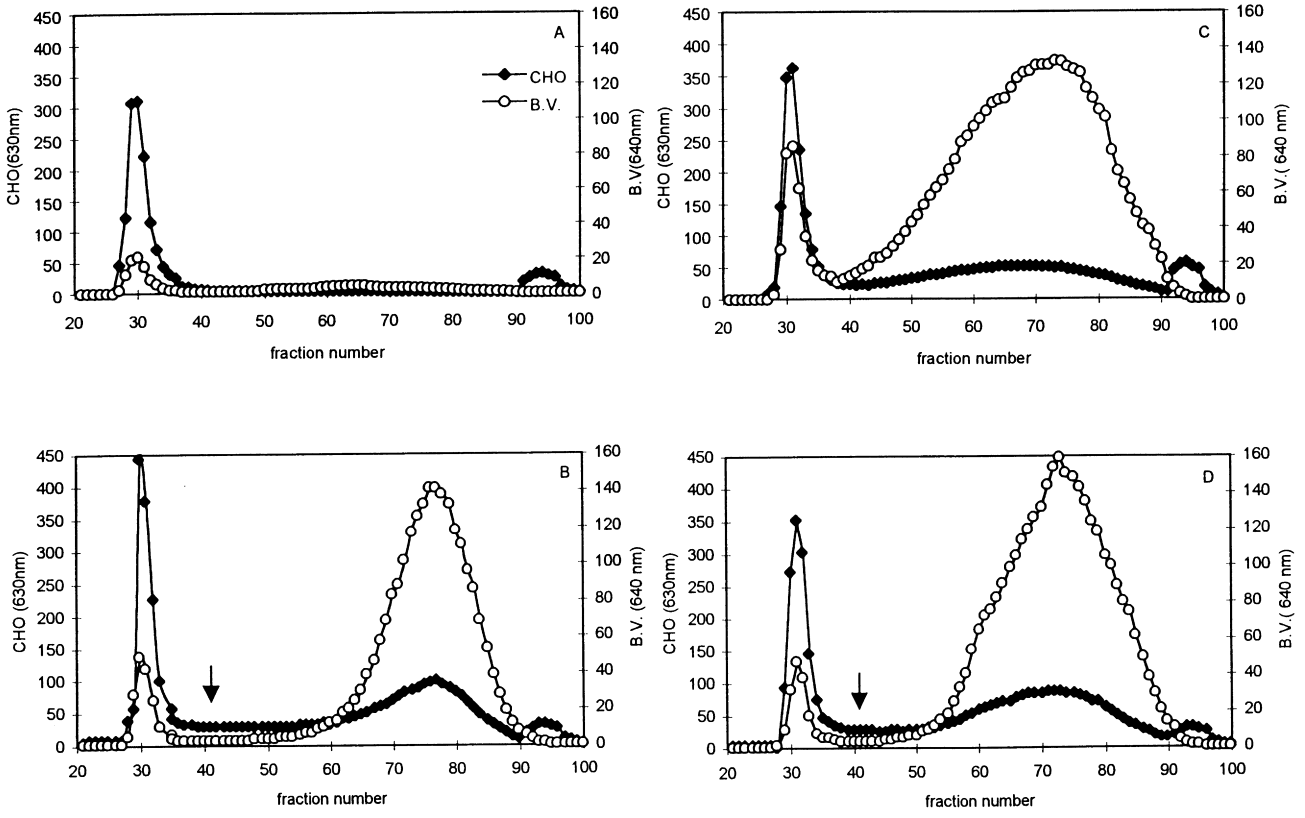

Fig. 3. Sepharose CL-2B gel permeation chromatographic profile of barley starches. CHO is the total carbohydrate, BV the blue value: (a) W.B. Merlin; (b) glacier; (c) high amylose glacier; (d) high amylose hull-less glacier. –◆–: total carbohydrate; –○–: blue value.

displaying substantial blue value corresponded to amylose. The last peak was glucose used as a marker. W.B. Merlin barley starch had a very small blue-value peak at around fraction number 65, indicating a small concentration of amylose content. High amylose glacier barley starch had a substantially broader second peak than the glacier and high amylose hull-less glacier starch samples, indicating that amylose had larger molecular weights and a broader molecular weight distribution.

Apparent and absolute amylose contents of barley starches were determined by using iodine potentiometric titration, and the results are shown in Table 1. Absolute amylose contents of W.B. Merlin, glacier, high amylose glacier, and high amylose hull-less glacier barley starches were 9.1, 29.5, 44.7, and 43.4%, respectively. W.B. Merlin amylopectin did not show any iodine affinity. GPC profiles of glacier (Fig. 3(b)) and high-amylose hull-less glacier (Fig. 3(d)) barley starches displayed molecules with smaller molecular-weight than amylopectin and little blue values (Fig. 3, marked with an arrow) indicating the molecules having short branches. This structural feature showed that these two barley starches consisted of intermediate component that had branched structures but smaller molecular weight than amylopectin (Kasemsuwan et al., 1995).

Chemical analysis showed that W.B. Merlin had 0.024% phosphorus; glacier, 0.052%; high amylose glacier, 0.057%; and high amylose hull-less glacier, 0.073% (Table 1). ^{31}P-NMR spectra (Fig. 4) showed signals mainly at the chemical shift between 0 and 2 ppm, indicating that barley starches had phospholipids (Lim, Kasemsuwan & Jane, 1994). The phosphorus contents of phospholipids were 0.022, 0.048, 0.051, and 0.060%, corresponding to 0.36, 0.78, 0.79, and 0.97% of phospholipids (Morrison & Tester, 1992), in W.B. Merlin, glacier, high amylose glacier, and high amylose hull-less glacier barley starch, respectively. The spectra of the two high amylose barley varieties (Fig. 4(c) and (d)) showed a signal with chemical shift at ~3 ppm, which indicated inorganic phosphate (Kasemsuwan & Jane, 1996). In contrary to that reported by Morrison and Tester (1992), no signals with chemical shift at ~3 ppm were found in the spectra of W.B. Merlin and glacier barley starches (Fig. 4(a) and (b)). The signals of phospholipids in the two high-amylose varieties were substantially broader than those of W.B. Merlin and glacier starches, which could be resulted from complex formation between the phospholipids and amylose (Morrison et al., 1993). There was a small proportion of the phospholipids remained uncomplexed and displayed a sharp peak at ~1 ppm.

3.3. Branch chain-length distributions

The branch chain-length distributions of the four varieties

Table 1
Amylose and phosphorus contents of barley starches

Sample	Iodine affinity (%)		Amylose content (%)		Phosphorus content (%)			
	Starch	Amylopectin	Apparent[a]	Absolute[b]	Chemical method	^{31}P-NMR method		
					Total phosphorus	Phospholipids	Inorganic phosphate	Total phosphorus[c]
W.B. Merlin	1.82 ± 0.03	nd[d]	9.1	9.1	0.024	0.022 (0.36%)[e]	nd	0.022
Glacier	5.95 ± 0.01	0.08 ± 0.03	29.8	29.5	0.052	0.048 (0.78%)	nd	0.048
High Amylose Glacier	9.60 ± 0.42	1.20 ± 2.13	48.0	44.7	0.057	0.049 (0.79%)	0.002	0.051
High amylose hull-less glacier	9.30 ± 0.43	1.10 ± 1.82	46.5	43.4	0.073	0.060 (0.97%)	0.008	0.068

[a] Apparent amylose content (%) = $IA_{starch}/IA_{amylose} \times 100\%$.
[b] Absolute amylose content (%) = $(IA_{starch} - IA_{amylopectin})/(IA_{amylose} - IA_{amylopectin}) \times 100\%$.
[c] Total phosphorus content was the sum of the phosphorus contents of phospholipids and inorganic phosphate.
[d] Not detectable.
[e] Data in the parenthesis were phospholipid contents. Phospholipid contents = Phosphorus contents in the form of phospholipids × 16.16 (Morrison & Tester, 1992).

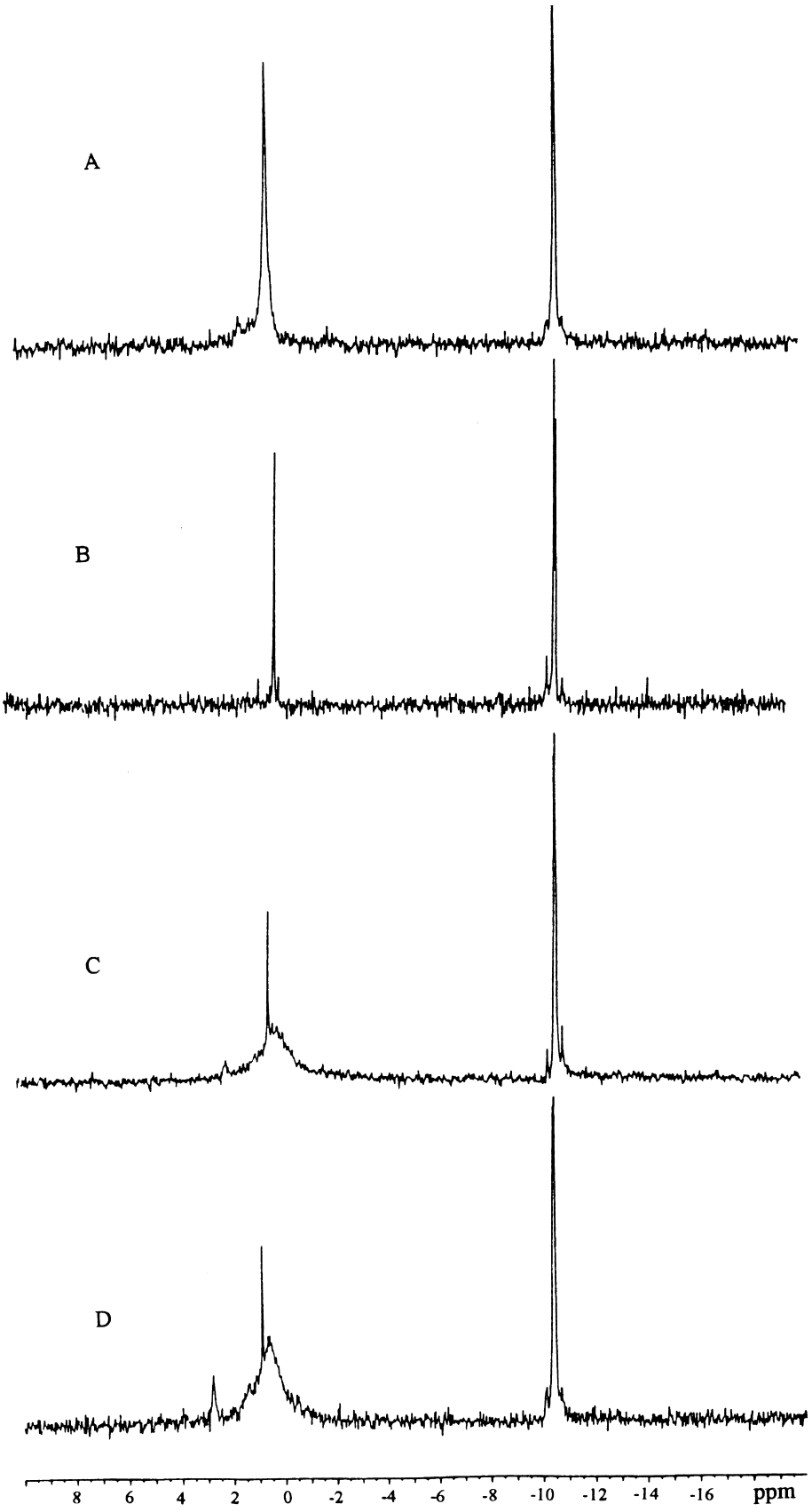

Fig. 4. ^{31}P nuclear magnetic resonance spectra of α-limited dextrin prepared from barley starches: (a) W.B. Merlin; (b) glacier; (c) high amylose glacier; (d) high amylose hull-less glacier. Signal at −10.37 ppm is an internal reference standard (nicotinamide adenine dinucleotide).

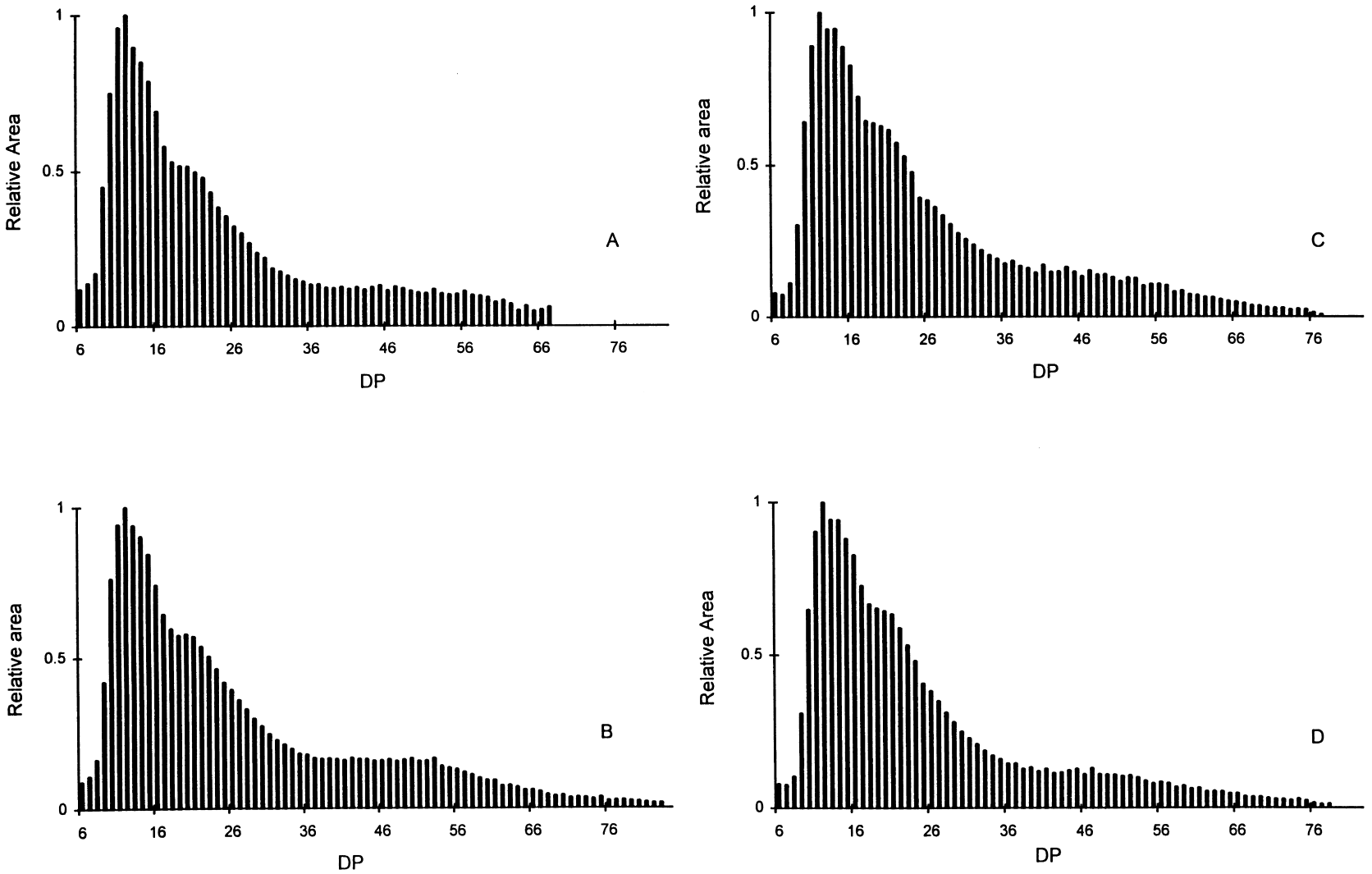

Fig. 5. Branch chain-length distributions of barley amylopectins by HPAEC-ENZ-PAD: (a) W.B. Merlin; (b) glacier; (c) high amylose glacier; (d) high amylose hull-less glacier.

Table 2
Summary of amylopectin structures of barley starches

Sample	Peak DP		Average	Distribution (%)					Highest detectable DP
	I	II	CL	DP6–9	DP6–12	DP13–24	DP25–36	DP ≥ 37	
W.B. Merlin	12	48	24.2	5.3	21.6	43.0	16.1	19.2	67
Glacier	12	50	26.6	4.1	18.0	40.9	17.2	23.7	82
High amylose glacier	12	48	25.5	3.1	16.5	44.9	17.9	20.7	79
High amylose hull-less glacier	12	48	24.5	3.2	17.4	47.5	17.2	17.8	78

of amylopectins debranched by isoamylase and analyzed by HPAEC-ENZ-PAD are shown in Fig. 5. These barley starch amylopectins had similar chain length distributions. Amylopectin of W.B. Merlin barley starch had its longest detectable chain of DP 67, which was the shortest among the four (Table 2). This lack of very long chains (DP > 67) resulted in no detectable iodine affinity in the amylopectin. This result agreed with those reported by Jane, Chen, Lee, McPherson, Wong and Radosavljevic, 1999. Amylopectins of the high amylose varieties had similar peak chain lengths as the others (DP 12 and 48 for peak I and II, respectively) but had lower proportions of DP 6–12, especially for the very short chains (DP 6–9). In contrast to the high-amylose maize starch that had very large proportions (26.1 and 29.5% for high-amylose maize V and VII, respectively) of long branch chains (DP > 37) (Jane et al., 1999), high amylose glacier and high amylose hull-less glacier barley starches had only

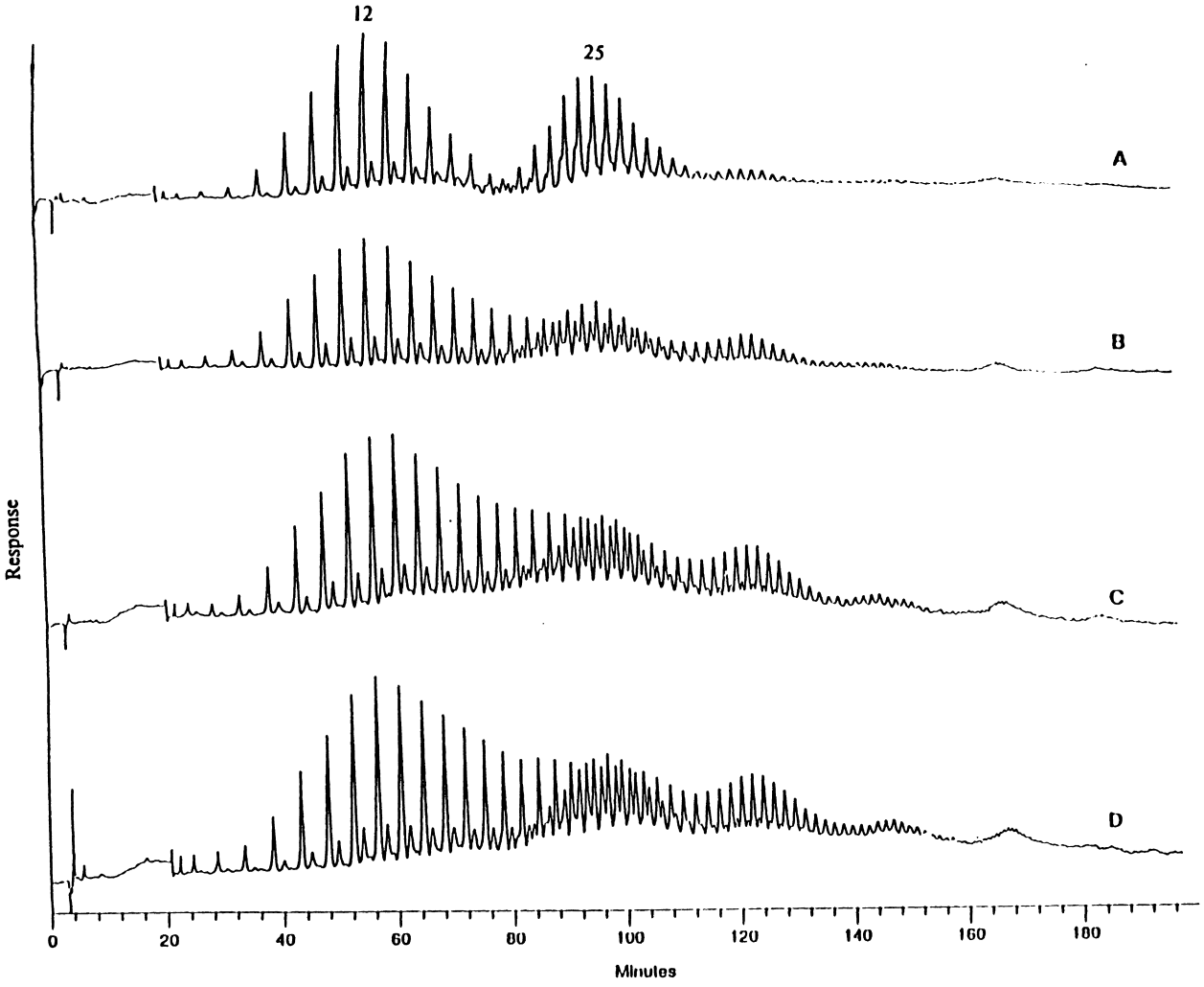

Fig. 6. HPAEC-ENZ-PAD chromatograms of barley Naegeli dextrins (after 12 days hydrolysis): (a) W.B. Merlin; (b) glacier; (c) high amylose glacier; (d) high amylose hull-less glacier.

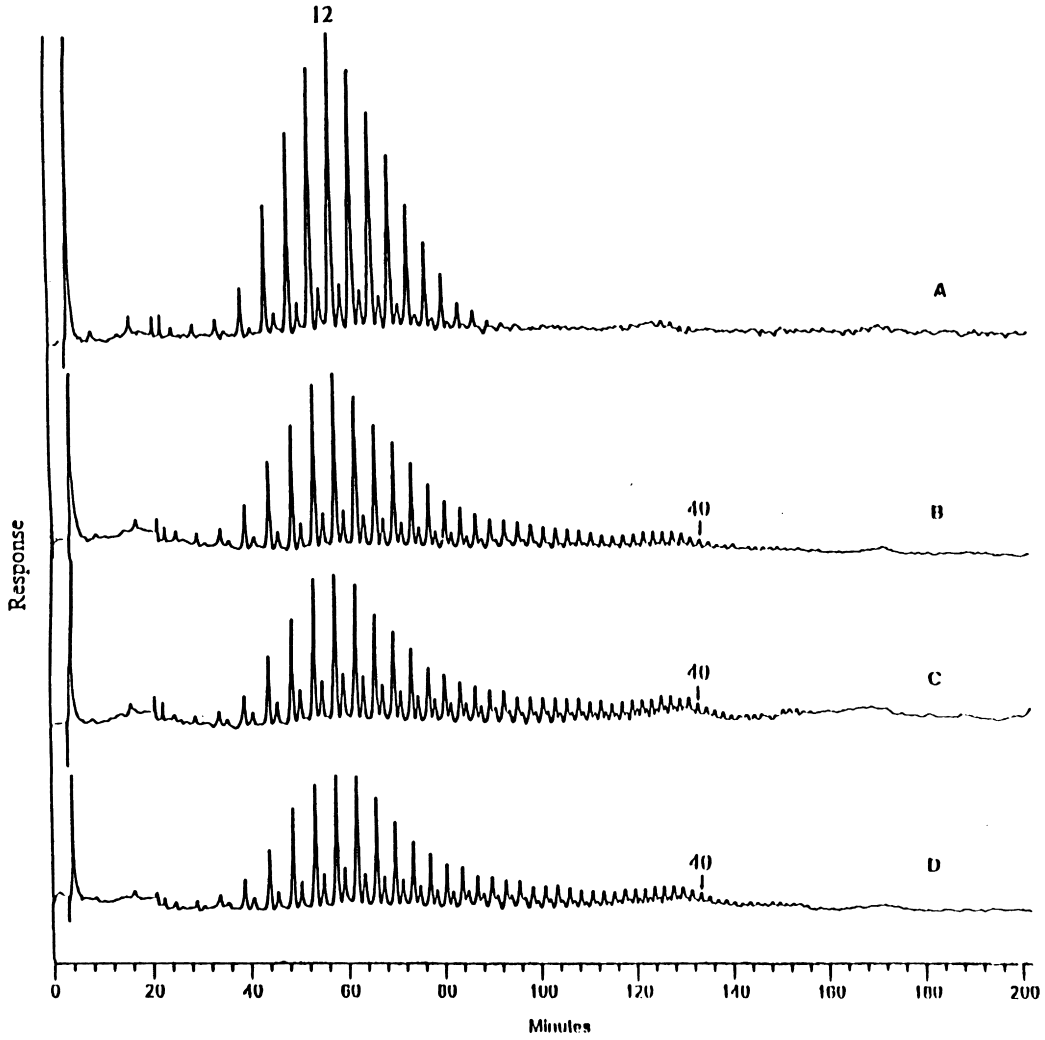

Fig. 7. HPAEC-ENZ-PAD chromatograms of debranched barley Naegeli dextrins (after 12 days hydrolysis): (a) W.B. Merlin; (b) glacier; (c) high amylose glacier; (d) High amylose hull-less glacier.

20.7 and 17.8% long branch chains of DP $>$ 37, similar to W.B. Merlin and glacier.

3.4. Naegeli dextrins

After 12 days of acid hydrolysis, 87% of the W.B. Merlin barley starch, 67% of the glacier barley starch, 71% of the high amylose glacier barley starch, and 66% of the high amylose hull-less glacier barley starch were hydrolyzed. It is plausible that W.B. Merlin barley starch, a waxy variety, swelled to a larger extent than other varieties and was more susceptible to acid hydrolysis, which resulted in a larger percentage hydrolysis.

Anion exchange chromatograms of the Naegeli dextrins obtained after 12 days of hydrolysis are shown in Fig. 6. For W.B. Merlin Naegeli dextrins, peaks were well separated, with clusters of peaks evident around DP 12 and 25 plus additional minor peaks at higher DP. Those clusters were also seen for the other varieties, but were not as well separated, and a cluster around DP 35 was readily visible. The peaks, at DP 12, 25, and higher DP, corresponded to linear, singly branched, double branched, and more highly branched molecules in the dextrin, respectively. After debranching with isoamylase (Fig. 7), W.B. Merlin contained only the cluster with a peak DP of 12; the other dextrins were also predominantly DP 12, but also contained significant fractions up to DP 40, which could be attributed to resistant fragments of retrograded amylose. Retrograded amylose contains crystalline and amorphous regions, and the crystalline region is resistant to acidic and amylolytic hydrolysis (Jane & Robyt, 1984; Lu, Jane & Keeling, 1997). The results showed that compared with potato and maize counterparts, barley amylopectins had larger proportions of α-1,6-branch linkages located within the crystalline region, which were protected from acid hydrolysis (Jane, Wong & McPherson, 1997). Potato Naegeli dextrin consists primarily of linear molecules, whereas normal and waxy maize

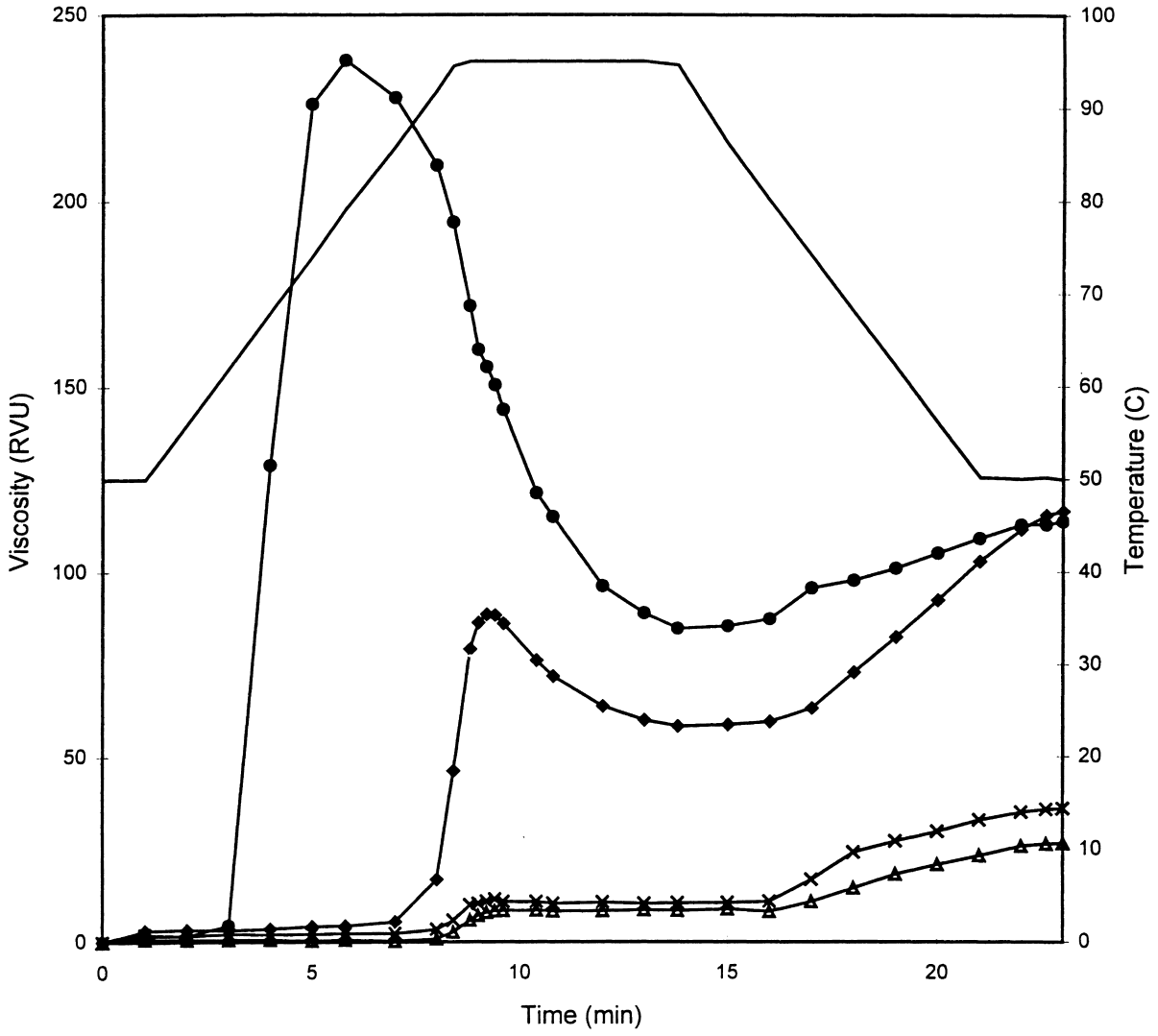

Fig. 8. Pasting profile of barley starches measured by Rapid Visco-Analyzer. —, temperature; ●, W.B. Merlin; ◆, glacier; ×, high amylose glacier; △, high amylose hull-less glacier.

Naegeli dextrins consist mainly of linear and singly branched molecules.

3.5. Thermal properties

Differential scanning calorimetric (DSC) thermal properties of the barley starches are shown in Table 3. The gelatinization onset temperatures of barley starches varied from 55.0 to 56.5°C. In contrast to their maize starch counterparts, high amylose barley starches did not show significantly higher gelatinization temperatures than did W.B. Merlin and glacier barley starches. High-amylose maize V and VII starches both display much higher gelatinization onset temperature (71.0 and 70.6°C, respectively), than waxy and normal maize starch (64.2 and 64.1°C, respectively) (Jane et al., 1999). This difference could be attributed to the fact that high-amylose maize starches had much longer branch chain-lengths than waxy and normal maize starches. The longer branch chains of high-amylose maize amylopectin developed into larger crystallites, which required higher temperatures to gelatinize. All the barley starch varieties had relatively short branch chain length. The shoulder of the branch chain length distribution (DP 18–21) (Fig. 5), equivalent to distances of 63–73.5 nm, which are in the proximity of the distance of the crystalline region of amylopectin (6.65 nm) (Cameron & Donald, 1992). The low proportion of DP 18–21, as shown in the shoulder, suggested a defective crystalline structure. The defective structure might be responsible for the very low gelatinization temperature of barley starch (Jane et al., 1999). Retrogradation of these starches after storage at 4°C for seven days was also analyzed by DSC. High amylose barley starches displayed higher retrogradation rates (Table 3), which could be due to the crystallization involving amylose molecules.

Table 3
Thermal properties of barley starches

Sample[a]	Native starch			Retrograded starch[b]			
	T_0 (°C)	T_p (°C)	ΔH (J/g)	T_0 (°C)	T_p (°C)	ΔH (J/g)	%Rt.
W.B. Merlin	55.4 ± 0.0[c]	60.3 ± 0.1	13.0 ± 0.2	38.3 ± 0.0	47.8 ± 0.0	3.1 ± 0.2	23.6
Glacier	55.0 ± 0.0	59.0 ± 0.0	9.2 ± 0.1	38.9 ± 0.5	48.7 ± 1.3	3.3 ± 0.2	35.6
High amylose glacier	55.5 ± 0.2	62.8 ± 0.0	7.7 ± 0.5	38.3 ± 0.6	49.2 ± 0.7	6.0 ± 0.1	78.5
High amylose hull-less glacier	56.5 ± 0.1	63.2 ± 0.0	7.3 ± 0.0	39.4 ± 0.2	49.7 ± 0.0	5.1 ± 0.1	70.1

[a] Samples (~2 mg, dry starch basis) and distilled water (~6 mg) were used for analysis T_0, T_p, and ΔH = *onset*, peak gelatinization temperature, and enthalpy change, respectively.
[b] After storage at 4°C for 7 days.
[c] Values were calculated from three replicates.

Table 4
Pasting properties of starches

Sample[a]	Pasting temperature (°C)	Peak viscosity (RVU)	Final viscosity (RVU)	Setback (RVU)
W.B. Merlin	61.8	221	102	32
Glacier	90.8	77	106	61
High amylose glacier	*	5	23	24
High amylose hull-less glacier	*	5	16	18

[a] Starch sample suspensions were 8%, w/w, on dry starch basis, in 28 g total weight.

3.6. Pasting property

RVA amylographs of the barley starch varieties showed distinctive differences in their pasting properties (Fig. 8). W.B. Merlin barley starch had a higher peak viscosity (221 RVU, compared with 205 RVU of waxy maize), and a lower pasting temperature (61.8°C, compared with 69.5°C of waxy maize) (Jane et al., 1999). A similar result was found in zero amylose hull-less barley starch (Zheng, Han & Bhatty, 1998). Glacier barley starch had a pasting temperature at 90.8°C, a lower peak viscosity of 77 RVU, and a substantially higher setback of 61 RVU. Two high-amylose barley starches showed similar pasting temperatures and very low peak viscosities (~5 RVU) (Table 4). The characteristic difference between barley starch varieties can be attributed to their amylose and phospholipid contents. With little amylose and being primarily amylopectin (>90%), W.B. Merlin barley starch could swell more freely and develop large peak viscosity at a low pasting temperature. Glacier barley starch contained 29.5% absolute amylose and 0.048% phosphorus corresponding to 0.78% phospholipids. Phospholipids could form helical complex with amylose and restricted granule swelling to a lower peak viscosity at a substantially higher pasting temperature. The high-amylose glacier barley starch, containing 43.4–44.7% absolute amylose and 0.049–0.060% phosphorus corresponding to 0.79–0.97% phospholipids, displayed the lowest peak viscosity. The very low granule swelling and viscosity were attributed to low amylopectin contents and amylose–lipid complexes. Amylopectin is primarily responsible for granule swelling (Tester & Morrison, 1990).

4. Conclusion

Four varieties of barley starches consisted of different proportions of amylose contents (9.1–44.7%), and total phosphorus contents (0.022–0.068%) that were mainly from phospholipids (0.36–0.97%). All the barley starch varieties had short branch chain lengths. Unlike high-amylose maize starch, high-amylose barley starches, as well as other varieties in this study, had low gelatinization temperatures. W.B. Merlin barley starch had a high peak viscosity, whereas high-amylose barley starches had very low peak viscosity.

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