Carbohydrate Research, 242 (1993) 203–215
Elsevier Science Publishers B.V., Amsterdam

Characterisation of hot-water-soluble components of starches

Gurunathan Murugesan *, Kiyoshi Shibanuma and Susumu Hizukuri †

Department of Biochemical Science and Technology, Kagoshima University, Kagoshima 890 (Japan)

(Received March 28th, 1992; accepted September 30th, 1992)

ABSTRACT

Starches of maize and wheat (cereals), potato (tuber), and sweet potato (rhizome) were extracted by 6 mM phosphate buffer (pH 6.2) above the gelatinisation temperatures, and fractionated into amylose (1-butanol precipitate) and amylopectin (supernatant solution). The amylose fraction was a mixture of linear and branched molecules and there were more branches in the amyloses from potato and sweet-potato amylose than in those from maize and wheat. The preponderant branches were short and probably clustered around the reducing terminal of the molecule. The amylose molecules in potato and sweet potato were larger than those in maize and wheat. The amylopectin fractions from potato and sweet potato showed some similarities in properties with their normal counterparts, whereas those of maize and wheat differed remarkably and contained considerable proportions of an unusual and novel fraction with dp 240 and 215, respectively, which may reflect the conditions of extraction and the botanical origin of the starch.

INTRODUCTION

Starch, by conventional definition, is composed essentially of linear amylose and branched amylopectin components. However, some intermediate structures that have properties of both amylose and amylopectin have been documented[1–3]. The structures and proportions of these components seem to depend on the conditions of isolation, extraction, and fractionation, on the botanical origin, on the stage of development at the time of isolation, and on other associated factors[1]. However, those components have not been characterised fully. It has been established that extensively purified amyloses from rice[4], maize[5], and other sources[6] are mixtures of linear and slightly branched materials, and some rice amylopectins have fairly long amylose-like chains[7–9]. These long chains appear to be a characteristic of various starches. Recently, hot-water extraction has been used in an attempt to

* Present address, The Cleveland Clinic Foundation, Cleveland, OH, USA.
† Author for correspondence.

obtain the linear molecules from wheat and potato starch[10]. We now report on further attempts to isolate the multiple molecular species from the starches of maize, wheat, potato, and sweet potato by extraction with hot water.

EXPERIMENTAL

Materials.—Starches from maize, wheat, and sweet potato were obtained commercially, and that from potato (var. *Kon'iku* 18) was kindly donated by Dr. S. Yoshioka (Hokkaido Agricultural Experimental Station).

Preparation of hot-water-soluble components.—Each starch (maize and wheat, 40 g; potato and sweet potato, 20 g) was washed with water and then collected by centrifugation. An aqueous suspension of each starch in 6 mM phosphate buffer (200 mL, pH 6.2) was poured slowly into the same buffer (1.8 L) at 85°C (maize and wheat), 70°C (potato), or 75°C (sweet potato) with mild stirring under N_2 and extracted for 1 h. Each suspension was centrifuged (8000*g*, 10 min), and a portion of the sediment was mixed with EtOH, collected on a G4 glass sinter, washed with EtOH and ether, and dried over $CaCl_2$ under reduced pressure for 1–2 days, to give the hot-water-insoluble starch. The above supernatant solution was treated with 1-butanol (190 mL), stored for 24 h at 30°C, then centrifuged (8500*g*, 10 min), to give the butanol-precipitate (amylose fraction). The supernatant solution was concentrated under reduced pressure, then freeze-dried to give the amylopectin fraction, which was dissolved in hot water; the solution was dialysed against water at 50°C, then freeze-dried. A suspension of each amylose fraction in water-saturated 1-butanol (WSB, 200 mL) was centrifuged (8000*g*, 10 min), and the sediment was recrystallised by dissolution in WSB (500 mL) at 85°C under N_2 and then cooling to room temperature. The precipitate was recovered by centrifugation (8000*g*, 10 min), washed with EtOH, collected on a G4 glass sinter, washed with EtOH and ether several times, then dried over $CaCl_2$ under reduced pressure (1–2 days). The outline for the preparation of various fractions is shown in Fig. 1.

Analytical methods.—The gelatinisation temperature (GT) range and GT were determined by the routine Congo-Red staining and birefringence end point (BEPT) methods. The temperature at which >95% of the granules stained or lost birefringence was taken as the GT. Iodine affinity, blue value, and absorption maximum were determined as described[11]. The number-average chain length ($\overline{cl}_n$) was determined by the modified Smith-degradation method[12,13] and by isoamylolysis[11]. Reducing sugar was determined by the Somogyi method[14] (with extended heating time and the Nelson[15] reagent) and by the modified Park–Johnson method[12]. Total carbohydrate was determined by the phenol–H_2SO_4 method[16]. Beta-amylolysis, isoamylolysis, and simultaneous hydrolysis with pullulanase and beta-amylase were carried out as described[11,12].

The weight-average dp ($\overline{dp}_w$) and dp distribution of amylose and its isoamylolysates were determined by gel-permeation HPLC on columns of TSK-GELs G6000PW, G4000PW, and G3000PW (Tosoh) connected in series and using a

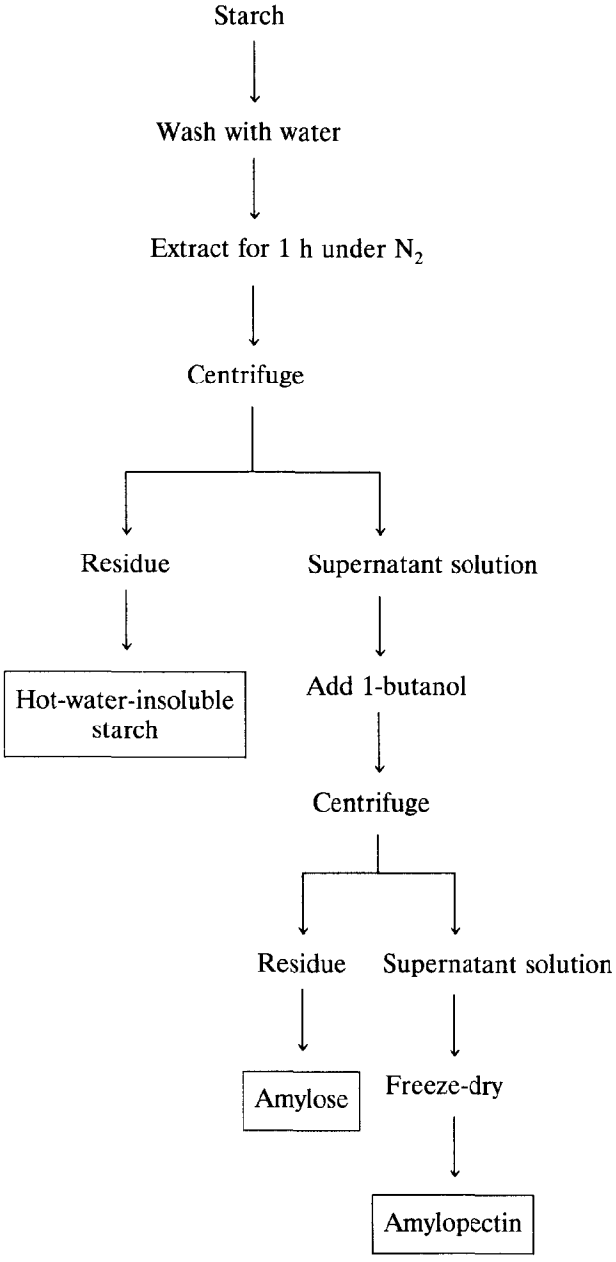

Fig. 1. The preparation of hot-water-soluble components of starches.

differential refractometer RI-8011 (Tosoh) and an LS-8 low-angle laser-light-scattering (LALLS) photometer (Tosoh) as detectors, as described[17,18].

The chain-length distribution of debranched amylopectins was analysed by

gel-permeation HPLC monitored with a LALLS photometer LS-8000 (Tosoh) and a differential refractometer as described[19], but with three columns connected in the following order: Asahipak GS-320 (7.6 × 500 mm) × 2 and TSK-GEL G3000PW (7.6 × 600 mm). The instrumental constant was determined using a standard pullulan (p-10, 10200 Da; Hayashibara Biochemical Laboratories).

High-performance anion-exchange chromatography (HPAEC) of debranched amylopectins was carried out with a Dionex BioLC Model 2000i system and a Model PAD II pulsed amperometric detector consisting of an amperometric flow-through cell with a gold working electrode, a silver–silver reference electrode, and a potentiostat[20]. The following pulse potentials and durations were used at range 2 (sampling period, 200 ms): E_1 0.10 (t_1 300), E_2 0.60 (t_2 120), E_3 −0.80 V (t_3 300 ms). The response time of the PAD II detector was set to 1.0 s. A Dionex CarboPac PA-1 column (250 × 4 mm i.d.) with a guard column (15 × 4 mm i.d.) was used. Eluent *A* was 150 mM NaOH prepared from carbonate-free aq 50% NaOH in 18 MΩ · cm NaOH deionised water, and eluent *B* was 150 mM containing 500 mM NaOAc (ref. 20). The gradient program was as follows: % of eluent *B*, 40 at 0 min, 50 at 10 min, 60 at 25 min, 70 at 40 min, 80 at 70 min. A solution of the debranched amylopectin (3 mg) in M NaOH (0.2 mL) was made up to 1 mL with deionised water and an aliquot (25 μL) was analysed.

RESULTS AND DISCUSSION

Extractability.—The conditions of extraction and the extractability of various starches and their fractions are shown in Table I. For a single-point characterisation, a temperature of 85°C is appropriate. However, at this temperature, the starches from potato and sweet potato formed gels, therefore they were extracted just above their GTs.

More starch (14–15%) was extracted from potato and sweet potato at 70–75°C than from cereals at 85°C (11%). As is well-known, the amylose (8.6–13.3%) was extracted more readily than the amylopectin (1.1–1.8%) (Table I), and the starch

TABLE I

Extractability of various starches and their fractions

Material	GT Range (°C)	GT (°C)	Extraction temperature (°C)	Total (%) extracted as				Total soluble (%)
				Insoluble	Amylose	Amylopectin	Loss [a]	
Maize	64–72	70	85	89.4	8.7	1.8	0.1	10.6
Wheat	54–66	64	85	89.5	8.6	1.8	0.1	10.5
Potato	60–70	68	70	84.8	13.3	1.1	0.8	15.2
Sweet potato	62–76	74	75	86.5	11.7	1.1	0.7	13.5

[a] Loss in the supernatant solutions discarded at various stages. All the data are on a dry basis.

TABLE II

General properties of various starches and their fractions

Properties	Maize	Wheat	Potato	Sweet potato
Iodine affinity (g/100 g)				
Native starch	3.3	3.9	3.3	3.3
Insoluble starch	2.1	2.7	1.1	1.4
Amylose	19.2	19.4	18.9	17.7
Amylopectin	2.3	4.9	1.0	trace
Blue value				
Native starch	0.351	0.383	0.388	0.367
Insoluble starch	0.254	0.264	0.249	0.238
Amylose	1.289	1.319	1.323	1.320
Amylopectin	0.341	0.402	0.219	0.150
λ_{max} (nm)				
Native starch	598	612	600	600
Insoluble starch	581	590	570	578
Amylose	635	649	650	647
Amylopectin	592	600	568	559

granules of tubers and rhizomes were disintegrated more easily than those of cereals.

General properties.—Table II shows some of the general properties of the starches and their fractions. The iodine affinities, blue values, and absorption maxima of the iodine-stained solutions of native starches were similar. These values were the highest for amylose, lowest for amylopectin, and intermediate for the insoluble fractions except for maize and wheat amylopectins (see below). The properties of amylose fractions were similar to those cited in the literature[3]. The recovery data for iodine affinity and blue value for various fractions shown in

TABLE III

Recovery data for iodine affinity and blue value of various starches and their fractions [a]

Material	Native starch	Distribution in various fractions				Recovery (%)
		Insoluble	Amylose	Amylopectin	Total	
Iodine affinity (g/100 g)						
Maize	3.3	1.88	1.67	0.04	3.59	109
Wheat	3.9	2.42	1.67	0.09	4.18	107
Potato	3.3	0.94	2.51	0.01	3.46	105
Sweet potato	3.3	1.22	2.07	trace	3.29	100
Blue value						
Maize	0.351	0.228	0.112	0.006	0.346	99
Wheat	0.383	0.237	0.113	0.007	0.357	93
Potato	0.388	0.213	0.176	0.002	0.391	101
Sweet potato	0.367	0.208	0.155	0.002	0.365	99

[a] Loss in the supernatant solution is accounted for in the insoluble fraction.

Table III are in good agreement with extractability (Table I) and general properties (Table II).

Molecular properties of the amylose fractions.—These properties are summarised in Table IV. The beta-amylolysis limits were in the range 79–85%, with slightly higher values for maize and wheat. Amylose of maize and wheat appeared to comprise smaller molecules than those of potato and sweet potato as indicated by $\overline{dp}_n$ (Table IV). However, the number-average chain lengths ($\overline{cl}_n$) had similar values in the range 195–240. The average number of branches per molecule ($\overline{B}_n$) of amylose was much lower for maize and wheat (2–3) than for potato (18) and sweet potato (11), and reflects a difference between cereals and root vegetables.

After debranching with isoamylase, the beta-amylolysis was incomplete as reported[12], and was 86% for potato and 90–95% for the others. $\overline{dp}_n$ of the debranched amylose was ~ 50% of the original value for maize and wheat, ~ 10% and ~ 15% for potato and sweet potato, respectively, and indicated that the amylose still had residual branches (0.7–1.5/molecule; Table IV). The number of branches differed considerably between cereals and others, but the isoamylase-resistant linkages per molecule were similar (0.7–1.5). Each amylose fraction was hydrolysed completely by the simultaneous action of pullulanase and beta-amylase, implying the isoamylase-resistant linkage was α-(1 → 6).

The beta-amylolysis limits and the number of branches in the native and debranched amyloses suggest that the branching could be near to, and clustered around, the reducing-end of the molecule, especially for potato and sweet potato (see below).

The distributions of molecular weights of the amyloses and their isoamylolysates by gel-permeation HPLC are shown in Figs. 2 and 3, respectively, and the data are summarised in Table V. Essentially, each fraction gave a single peak, with a small

TABLE IV

Properties of the amylose fractions and their isoamylolysates

Properties	Maize	Wheat	Potato	Sweet potato
Amylose				
Beta-amylolysis limit (%)	83	85	82	79
$\overline{Dp}_n$	650	900	4470	2570
$\overline{Cl}_n$	195	225	240	220
$\overline{Nc}$	3.3	4.0	18.6	11.7
$\overline{B}_n$ [a] (A)	2.3	3.0	17.6	10.6
Isoamylase-resistant linkage [b] (%)	35	50	5.7	6.6
Isoamylolysate				
Beta-amylolysis limit (%)	90	94	86	95
$\overline{Dp}_n$	345	555	470	375
$\overline{Nc}$	1.8	2.5	2.0	1.7
$\overline{B}_n$ [a] (B)	0.8	1.5	1.0	0.7

[a] Average number of branch linkages per molecule. [b] B/A × 100.

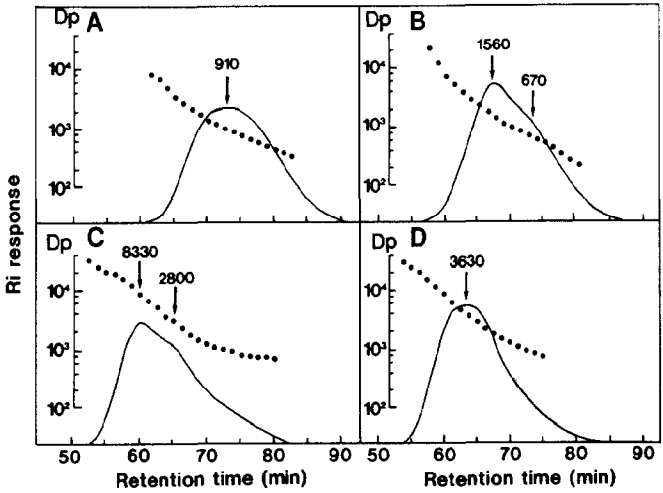

Fig. 2. Gel permeation HPLC (TSK-GEL G6000PW, G4000PW, and G3000PW) of amylose fractions from various starches: ———, refractive index (Ri) response; •, dp; ↓, dp values; A, maize; B, wheat; C, potato; D, sweet potato.

shoulder for wheat and potato (Fig. 2). The apparent dp distributions of these amyloses, especially of maize and wheat, showed that the large molecules were considerably smaller than those of whole amyloses [maize 400–14 700 (ref. 21), wheat 360–15 600 (ref. 22), sweet potato 840–19 100 (ref. 17), and potato 840–21 800 (ref. 17), suggesting that the fractions of larger molecular weight remained in the insoluble residues as reported previously[1]. These large amylose molecules apparently control the swelling of the granules during gelatinisation.

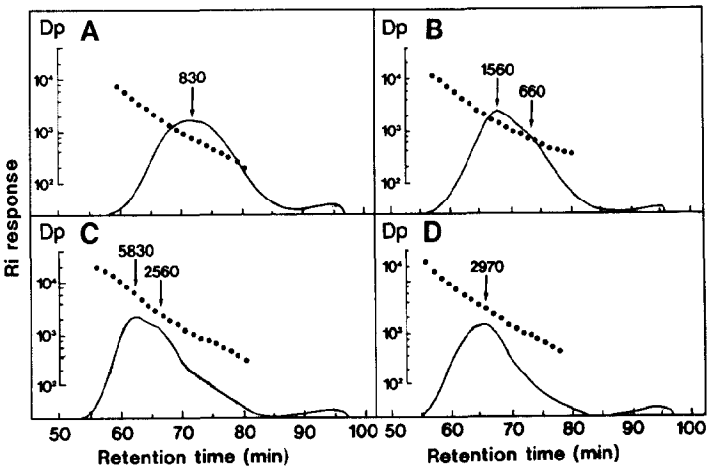

Fig. 3. Gel permeation HPLC (TSK-GEL G6000PW, G4000PW, and G3000PW) of isoamylolysates of amylose fractions from various starches. For key, see Fig. 2.

TABLE V

Dp distributions of amylose fractions and their isoamylolysates

	Maize	Wheat	Potato	Sweet potato
Amylose				
$\overline{Dp}_w$	1100	1610	6110	4610
Peak dp	910	1560	8330	3630
Shoulder dp		670	2800	
Apparent dp distribution [a]	370–2200	340–4500	990–15000	870–11000
Isoamylolysate				
$\overline{Dp}_w$	1030	1570	4280	3310
Peak dp	830	1560	5830	2970
Shoulder dp		660	2560	

[a] Dp values of the 10% (by weight) lowest and highest molecular weights.

Isoamylase reduced the whole, peak, and shoulder dp values (Figs. 2 and 3, and Table V) and produced a small proportion of material with very low $\overline{dp}_w$ at the end of the chromatogram (Fig. 3) and an extended retention time on the column. The decrease in molecular weight during isoamylolysis was low for maize and wheat but appreciable for potato and sweet potato, and suggests that short branches are associated with the former and longer branches with the latter. A multi-branched nature seems more probable, as revealed from the elution profile (see data for potato and sweet potato in Fig. 3) which otherwise might have given at least another shoulder, if not a peak, with still higher $\overline{dp}_w$ rather than a relatively flat and extended peak at the end of the chromatogram. These observations support the hypothesis[5] that small chains contribute to the branching and form clusters near the reducing end of the amylose molecules.

Molecular properties of the amylopectin fractions.—These properties are shown in Table VI. The beta-amylolysis limit was ~60% for the amylopectins of maize and wheat and ~53% for these of potato and sweet potato. After isoamylolysis, these values increased up to 90%, except for potato (85%) for which there was a

TABLE VI

Properties of the amylopectin fraction of various starches

Properties	Maize	Wheat	Potato	Sweet potato
Beta-amylolysis limit (%)	62	58	52	53
$\overline{Dp}_n$ (isoamylolysis) [a]	22	22	26	19
$\overline{Cl}_n$ (Smith degradation)	17	17	15	14
Isoamylolysate				
Beta-amylolysis limit (%)	91	90	85	90

[a] Extending the time or doubling the enzyme concentration had no additional effect on further debranching.

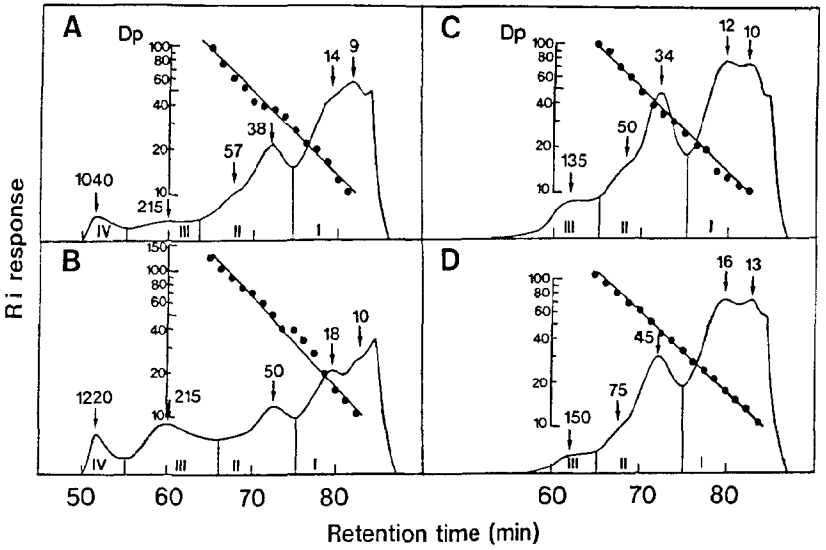

Fig. 4. Gel permeation HPLC [Asahipak GS-320 (×2) and TSK G3000PW] of isoamylolysates of "amylopectin" fractions from various starches. For key, see Fig. 2.

similar trend for the amylose fraction (Table IV). A striking feature is the substantial difference in the chain length as estimated by isoamylolysis and Smith degradation, especially for potato amylopectin. When the isoamylolysis was prolonged, and the concentration of the enzyme was increased, there was no further debranching. However, these materials were hydrolysed completely by the combined action of pullulanase and beta-amylase, so that the isoamylase-resistant linkage was α-(1 → 6).

The elution profiles of the debranched amylopectins on gel-permeation HPLC are shown in Fig. 4 and their chain-length distributions are summarised in Table VII. The debranched amylopectins of maize and wheat were resolved into four sub-fractions, whereas those of potato and sweet potato were resolved into three sub-fractions (Fig. 4). The maize and wheat amylopectins were eluted much earlier and contained a substantial proportion of long chains (sub-fraction IV, $\overline{cl}_w > 800$) which were absent in potato and sweet potato[21] (Fig. 4 and Table VII). Consequently, the overall average chain length was greater for maize (75) and wheat (129) than for potato (38) and sweet potato (34). Even if the sub-fractions IV of maize and wheat were omitted from the calculation, the resulting $\overline{cl}_w$ values were 42 and 72, respectively (Table VII) and were higher than those of potato and sweet potato. The $\overline{cl}_n$ of whole-potato amylopectin is larger than those of maize and wheat[23,24], and the $\overline{cl}_w$ of the water-extracted potato and sweet potato were also slightly higher than those of their normal amylopectins[25]. It has been suggested[26,27] that tuber amylopectin has longer B chains [carrying other chain(s) at their C-6 positions[28]] than cereal amylopectins. Conversely, the $\overline{cl}_w$ of the water-extracted

TABLE VII

Chain-length distributions of the amylopectins of maize, wheat, potato, and sweet potato

Material	Fraction				
	Whole [a]	I	II	III	IV
Maize					
$\overline{Cl}_w$	75 (42)	14	50	240	810
Weight (%)	100	59.0	29.5	7.3	4.2
Mole (%)	100	87.1	12.2	0.6	0.1
Wheat					
$\overline{Cl}_w$	129 (72)	18	60	215	1040
Weight (%)	100	50.1	23.3	20.8	5.8
Mole (%)	100	85.0	11.8	3.0	0.2
Potato					
$\overline{Cl}_w$	38	13	40	180	
Weight (%)	100	56.6	34.3	9.1	
Mole (%)	100	82.7	16.3	1.0	
Sweet potato					
$\overline{Cl}_w$	34	18	50	150	
Weight (%)	100	65.5	29.7	4.8	
Mole (%)	100	85.3	13.9	0.8	

[a] Figures in parentheses indicate the cl when fractions IV of maize and wheat were omitted from the calculation.

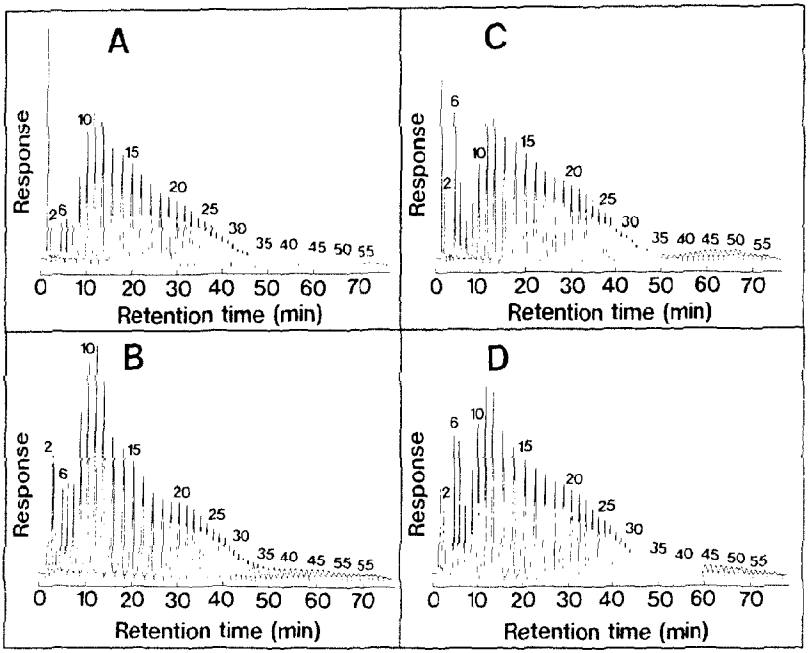

Fig. 5. HPAEC (CarboPac PA-1) of isoamylolysates of amylopectin fractions from various starches: peak numbers indicate the chain length; A, maize; B, wheat; C, potato; D, sweet potato.

TABLE VIII

Chain-length distributions of the debranched amylopectins of maize, wheat, potato, and sweet potato determined by hpaec

Chain length (dp)	Retention time (min)	Peak area (%)			
		Maize	Wheat	Potato	Sweet potato
2–5	2.1–3.6	0.8	2.7	0.9	1.3
6	4.5	0.8	1.6	2.8	2.3
7–9	5.6–8.5	4.8	8.3	3.8	6.3
10	10.1	4.9	6.1	3.0	4.0
11–14	11.8–18.1	24.6	24.9	21.8	23.5
15	20.4	5.8	5.0	5.4	5.1
16–19	22.5–28.9	18.3	15.2	17.9	17.1
20	30.7	3.7	3.3	4.0	3.6
21–24	32.4–37.1	11.9	11.2	13.1	12.0
25	38.5	2.1	2.0	2.4	2.4
26–29	39.9–43.4	5.7	5.8	6.7	6.8
30	44.5	0.9	1.0	1.1	1.2
31–34	45.7–49.1	3.0	2.7	2.9	3.5
35	50.3	0.6	0.6	0.5	0.6
36–39	51.5–55.1	2.8	2.0	1.9	1.8
40	56.3	0.8	0.5	0.5	0.5
41–44	57.5–61.0	2.6	1.9	2.6	2.0
45	62.2	0.7	0.5	0.7	0.6
46–49	63.4–66.7	2.4	1.9	3.0	2.2
50	67.8	0.6	0.4	1.0	0.4
> 50	69.9–76.1	2.2	2.4	4.0	2.8
Total		100	100	100	100

maize and wheat amylopectins had excessively larger $\overline{cl}_n$ and B chains than those of potato amylopectin. This finding is consistent with the view of Finch and Sebesta[29] that wheat amylopectin may have fewer, longer, inter-cluster chains than potato amylopectin. Evidently, these high $\overline{cl}_w$ values are due to considerably higher proportions of long-chain sub-fractions III and IV of the water-extracted maize and wheat amylopectins than those of the respective normal amylopectins[21,30] and also than those of potato amylopectin (Fig. 4 and Table VII). Amylopectins with unusual structures, even if present in only small proportions, could be characterised by degradation with cyclodextrin glycosyltransferase[27] and *Pseudomonas stutzeri* amylase[29], and a sequential degradation with beta-amylase, isoamylase, and beta-amylase[30].

Chromatograms of the debranched amylopectins on HPAEC are shown in Fig. 5, and the chain-length distribution and the relative molar distribution of chains of dp 2–17 are summarised in Tables VIII and IX, respectively. Significant proportions of chains of dp 2–5 were found because the smallest chain of normal amylopectins observed most often[20] has dp 6. Wheat had the highest proportion of these short chains followed by sweet potato, potato, and maize (Fig. 5 and Table

TABLE IX

Relative molar distribution of debranched amylopectin chains with cl 2–17

Cl (dp)	Relative PAD response [a]	Molar distribution (%)			
		Maize	Wheat	Potato	Sweet potato
2	0.32	1.9	3.8	3.7	2.8
3	0.43	0.7	4.9	0.6	1.4
4	0.54	0.9	2.0	1.0	1.5
5	0.65	0.8	1.3	0.6	1.2
6	0.74	2.8	4.3	9.6	6.8
7	0.82	2.6	4.5	4.6	6.4
8	0.89	2.8	5.0	2.6	4.0
9	1.00	7.2	8.6	3.9	5.9
10	1.10	10.8	11.2	7.2	8.5
11	1.20	11.7	11.4	9.7	10.3
12	1.31	11.9	10.1	10.7	11.2
13	1.38	11.2	8.6	11.0	10.3
14	1.46	10.2	7.6	10.5	9.0
15	1.55	9.1	6.5	9.1	7.7
16	1.59	8.2	5.6	8.2	6.8
17	1.65	7.2	4.6	7.0	6.2
Total		100	100	100	100

[a] On molar basis; adapted from refs 20 and 31.

VIII). In addition, the proportion of chains of dp 6 was high in potato and sweet potato compared to their typical amylopectins[20]. However, chains of dp > 50 could not be resolved into individual peaks by this technique mainly due to the low sensitivity of the detection and the limited proportion of each component.

REFERENCES

1 W. Banks and C.T. Greenwood, *Starch and its Components*, University Press, Edingburgh, 1975.
2 D.J. Manners, *Cereal Foods World*, 30 (1985) 461–467.
3 S. Hizukuri, *Denpun Kagaku*, 35 (1988) 185–198.
4 Y. Takeda, S. Hizukuri, and B.O. Juliano, *Carbohydr. Res.*, 186 (1989) 163–166.
5 Y. Takeda, T. Shitaozono, and S. Hizukuri, *Carbohydr. Res.*, 199 (1990) 207–214.
6 Y. Takeda, S. Hizukuri, C. Takeda, and A. Suzuki, *Carbohydr. Res.*, 165 (1987) 139–145.
7 Y. Takeda, S. Hizukuri, and B.O. Juliano, *Carbohydr. Res.*, 168 (1987) 79–88.
8 Y. Takeda, N. Maruta, S. Hizukuri, and B.O. Juliano, *Carbohydr. Res.* 187 (1989) 287–294.
9 S. Hizukuri, Y. Takeda, N. Maruta, and B.O. Juliano, *Carbohydr. Res.*, 189 (1989) 227–235.
10 S. Hizukuri, *Carbohydr. Res.*, 217 (1991) 251–253.
11 A. Suzuki, S. Hizukuri, and Y. Takeda, *Cereal Chem.*, 58 (1981) 286–290.
12 S. Hizukuri, Y. Takeda, M. Yasuda, and A. Suzuki, *Carbohydr. Res.*, 94 (1981) 205–213.
13 Y. Takeda, S. Hizukuri, and B.O. Juliano, *Carbohydr. Res.*, 148 (1986) 299–308.
14 M. Somogyi, *J. Biol. Chem.*, 195 (1952) 19–23.
15 N. Nelson, *J. Biol. Chem.*, 153 (1944) 375–380.
16 M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
17 S. Hizukuri and T. Takagi, *Carbohydr. Res.*, 134 (1984) 1–10.
18 T. Takagi and S. Hizukuri, *J. Biochem. (Tokyo)*, 95 (1984) 1459–1467.
19 S. Hizukuri, *Carbohydr. Res.*, 147 (1986) 342–347.

20 K. Koizumi, M. Fukuda, and S. Hizukuri, *J. Chromatogr.*, 585 (1991) 233–238.
21 Y. Takeda, T. Shitaozono, and S. Hizukuri, *Staerke*, 40 (1988) 51–54.
22 K. Shibanuma, Y. Takeda, and S. Hizukuri, unpublished data.
23 C.T. Greenwood, in W. Pigman and D. Horton (Eds.), *The Carbohydrates, Chemistry and Biochemistry*, Vol. 2B, Academic Press, New York, 1970, pp 471–513.
24 W.R. Morrison and J. Karkalas, in P.M. Dey (Ed.), *Methods in Plant Biochemistry*, Vol. 2, Academic Press, London, 1990, pp 323–352.
25 S. Hizukuri, *Carbohydr. Res.*, 141 (1985) 295–306.
26 S. Hizukuri, *Carbohydr. Res.*, 147 (1986) 342–347.
27 H. Bender, R. Siebert, and A. Stadler-Szoke, *Carbohydr. Res.*, 110 (1982) 245–259.
28 S. Peat, W.J. Whelan, and G.J. Thomas, *J. Chem. Soc.*, (1952) 4546–4548.
29 P. Finch and D.W. Sebesta, *Carbohydr. Res.*, 227 (1992) c1–c4.
30 S. Hizukuri and Y. Maehara, *Carbohydr. Res.*, 206 (1990) 145–159.
31 K. Koizumi, Y. Kubota, T. Tanimoto, and Y. Okada, *J. Chromatogr.*, 464 (1989) 365–373.