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Characterization of Lotus Starch

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ABSTRACT

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Lotus rhizome starch and its components were characterized by gel-exclusion high-performance liquid chromatography (HPLC) monitored with a low-angle laser-light-scattering photometer and a differential refractometer and by high-performance anion-exchange chromatography (HPAEC) monitored with a pulsed amperometric detector, as well as by conventional analyses. Amylose molecules were large, with number-average and weight-average degrees of polymerization (DPs) of 4,170 and 8,040, respectively, number-average chain length ($\overline{CL}$n) of 540, and apparent DP distribution range of 520–42,000. The $\overline{CL}$n of amylopectin was 22.3, and its distribution by gel-exclusion HPLC showed three peaks, at DP 14, 42, and 1,900, and a shoulder at DP 63. The relative chain length distribution between DP 6 and 17 analyzed by HPAEC and corrected by molar responses revealed that DP 8 and 13 were the minimum and maximum, respectively. The amylose content was 15.9% determined by iodine affinity and 17.4% determined by blue value. The onset of gelatinization determined by photopastegraphy was at 58.5°C. The amylogram of the starch resembled that of tapioca starch. The retrogradation tendency of lotus starch was slower than that of potato, lily, and kuzu starches but faster than that of tapioca starch.

Starches from different botanical sources have diverse physicochemical and functional properties. For both academic and industrial purposes, we are searching for starches with specific properties. Several root crops are interesting from this point of view because they store abundant starch with characteristic properties.

Indian lotus (*Nelumbo nucifera* Gaertn.) is cultivated in swamplands or paddy fields in Japan. Its flowers, seeds, and rhizomes are edible, and the rhizome is especially appreciated as a traditional food. The fresh rhizome contains about 15% starch but when steamed or boiled is not pasty in texture like potato, sweet potato, or taro. The starch is commercially available in China and seems to have some specific uses (Fujimoto et al 1985). Fuwa et al (1979), Sugimoto et al (1984), and Fujimoto et al (1985, 1988) have reported some of the physicochemical and morphological properties of lotus starch; however, the starch has not been thoroughly investigated yet. We characterized its molecular and functional properties in detail.

MATERIALS AND METHODS

Materials

Crystalline grade of isoamylase from *Pseudomonas amyloderamosa* was obtained from Hayashibara Biochemical Laboratories Ltd. (Okayama, Japan), and crystalline sweet potato β-amylase was prepared by the method of Takeda and Hizukuri (1969) and was recrystallized from aqueous ammonium sulfate solution. All chemicals of the highest grade available were purchased from Wako Pure Chemicals Ind. (Osaka, Japan). Maize, waxy maize, wheat, and tapioca starches were donated by Sanwa Starch Co. (Nara, Japan), and potato starch was prepared by a conventional method in the laboratory (Takeda et al 1983).

Preparation of Lotus Starch

Lotus rhizomes cultivated in Tokushima prefecture, Japan, were purchased in January 1988 from a local market. The rhizomes (4 kg) were peeled, sliced in small pieces, and then homogenized with ice-cold water in a home blender. The homogenate was squeezed through double-layered gauze by hand. The fibrous residue was again squeezed with ice-cold water. The combined extract was allowed to stand for several hours at 0°C. Starch was then recovered from the extract by decantation. The starch was washed several times by suspension in cold water and centrifugation at 3,300 × *g*. The final precipitate (crude starch, 193 g) was dried at about 5°C. The crude starch (50 g) was shaken with a mixture of water (200 ml) and toluene (100 ml) for 15 min. The starch was recovered from the resulting milky suspension by centrifugation at 3,300 × *g* for 10 min, and the toluene treatment was repeated to remove protein. The starch was washed once in ethanol and several times with distilled water by centrifugation. The thus purified starch was dried over calcium chloride under reduced pressure in a refrigerator (yield 158 g). The starch was defatted with hot 85% aqueous methanol (5 v/w) for 5 hr five times (Schoch 1942) and again dried in a refrigerator.

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Fractionation of Amylose and Amylopectin

The defatted starch (10 g) was dissolved in hot dimethyl sulfoxide solution, precipitated with ethanol to remove trace amount of lipids, which interfere with complete dispersion (Takeda et al 1986), and then fractionated into amylose and amylopectin under a nitrogen atmosphere by the method of Lansky et al (1949), as modified by Hizukuri et al (1981). The amylose fraction was recrystallized five times from aqueous 1-butanol solution (10%). The purity of the amylose was confirmed by gel permeation chromatography (Takeda et al 1984). The yields of amylose and amylopectin were 1.41 g and 7.58 g, respectively.

Temperature-Dependent Pasting Behavior

One gram (dry basis) of starch was suspended in 19 ml of water, and the suspension was heated in a water bath, which was thermostated at the desired temperature (±0.05°C), with gentle stirring for 20 min. The suspension was then poured into ethanol (100 ml) with stirring. The precipitate was filtered through a glass filter, washed with excess ethanol and ether, and then dried over calcium chloride in a desiccator under vacuum until it was analyzed.

Retrograding Behavior

One gram (dry basis) of starch was suspended in 19 ml of water, and the suspension was heated in a boiling water bath for 20 min. The paste was immediately transferred to an ice-cold bath, kept for a desired period (1–120 hr), and then powdered as described above by treatments with alcohol and ether and dried over calcium chloride in a desiccator until it was assayed.

General Analytical Methods

The procedures for measuring the blue value (absorbance at 680 nm of iodine-stained solution), iodine affinity (grams per 100 g), limiting viscosity number ([η]) (in 1M KOH, 22.5°C), and β-amylolysis limit (%, conversion to maltose) were as described by Suzuki et al (1981). Total and esterified phosphorus as glucose-6-phosphate were determined by the methods of Fiske and Subbarow (1925) and by using glucose 6-phosphate dehydrogenase after acid hydrolysis (Hizukuri et al 1970), respectively. Total carbohydrate was measured by the phenol-sulfuric acid method (Dubois et al 1956). Degree of gelatinization (DG) was determined by glucoamylase digestion (DG-g) and by iodine titration (DG-i), as described by Suzuki and Hizukuri (1979).

The reducing residues of amylose and debranched amylopectin were assayed by modifications of the Park-Johnson (Hizukuri et al 1981) and Somogyi-Nelson (Hizukuri et al 1970) colorimetric methods, respectively. The number-average degree of polymerization ($\overline{DP}$n) was calculated from the values of total carbohydrate and the reducing residue (Hizukuri et al 1981). The nonreducing residues of amylose and amylopectin were determined fluorometrically (Hizukuri et al 1981, Takeda et al 1984) and photometrically (Hizukuri and Osaki 1978) after Smith degradation, respectively. The number-average chain length ($\overline{CL}$n) of amylose and amylopectin was calculated from the number of nonreducing residues and total carbohydrate as glucose. The $\overline{CL}$n of amylopectin was determined also from the reducing value after debranching with *Pseudomonas* isoamylase (Hizukuri and Maehara 1990).

TABLE I
General Properties of Lotus Starch and Its Components

Property	Starch	Amylopectin	Amylose
Iodine affinity, g/100 g	3.37	0.22	20.2
Blue value	0.375	0.125	1.56
Amylose content, %	15.9[a] (17.4[b])		
β-Amylolysis limit, %	65	55	90
Phosphorus, organic, ppm	48[c] (53[d])	50	0
as glucose-6-phosphate, ppm		21	

[a] Calculated from iodine affinities of starch, amylose, and amylopectin.
[b] Calculated from blue values of starch, amylose, and amylopectin.
[c] Defatted.
[d] Purified with toluene.

Gel-Exclusion HPLC

The weight-average degrees of polymerization ($\overline{DP}$w) of amylose and the DP distributions were estimated by gel-exclusion high-performance liquid chromatography (HPLC) monitored with a low-angle laser-light-scattering (LALLS) photometer and a differential refractometer. A series of columns of TSK-gel G6000PW, G4000PW, and G3000PW (each 7.5 × 600 mm) was used as described previously (Hizukuri and Takagi 1984, Takagi and Hizukuri 1984). The chain length distribution of debranched amylopectin was estimated by HPLC-LALLS with a series of columns of an Asahipak GS-320 (7.6 × 500 mm), GC (7.5 × 7.5 mm), a TSK-gel G2000SW (7.5 × 600 mm), and GC (7.5 × 7.5 mm), instead of the three TSK-gel SW columns used in a previous experiment (Hizukuri 1986). The columns were eluted with 0.1M phosphate buffer (pH 6.0) containing 0.02% sodium azide at 0.4 ml/min. The column temperature was maintained at 35±0.1°C in a water bath with a thermostat. A sample solution (4.5 mg, 0.3 ml) prepared as described by Hizukuri and Maehara (1990) was injected into HPLC.

HPAEC with Pulsed Amperometric Detection

The chain length distribution of debranched amylopectin was analyzed also by a Dionex BioLC model 4000i system (Sunnyvale, CA) and a pulsed amperometric detector (PAD) with an amperometric flow-through cell with a gold working electrode, a silver-silver chloride reference electrode, and a potentiostat. The pulsed potentials and durations were $E_1 = 0.10$ V ($t_1 = 300$ msec), $E_2 = 0.60$ V ($t_2 = 120$ msec), and $E_3 = -0.80$ V ($t_3 = 300$ msec) at range 2 (sampling periods, 200 msec). The output at attenuation

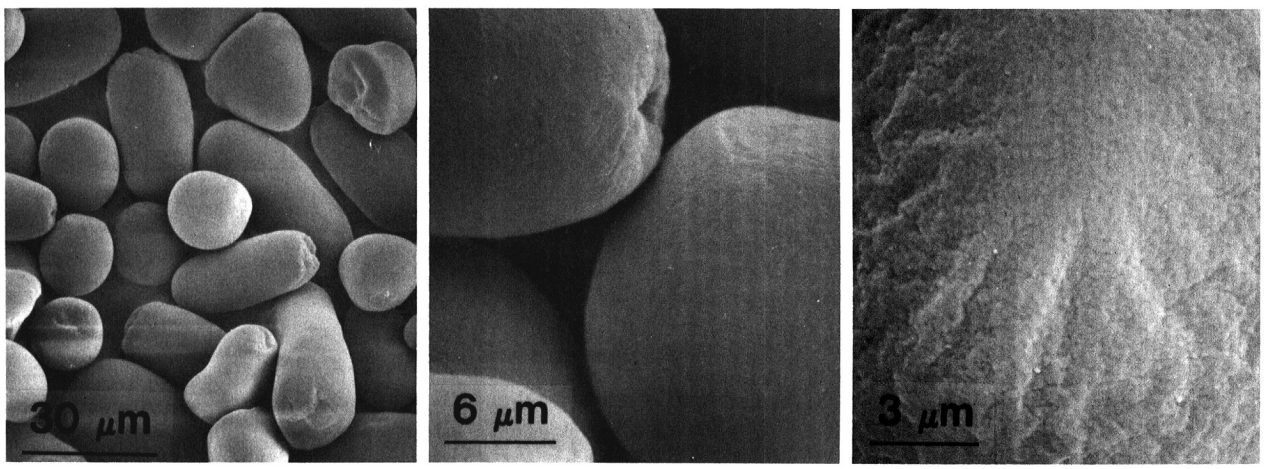

Fig. 1. Scanning electron micrographs of lotus starch.

10,000 was recorded by a C-R6A Chromatopac (Shimadzu, Kyoto, Japan) with attenuation 2 (4 mV/full scale) and chart speed 3 mm/min. A Dionex CarboPac PAI column (250 × 4 mm) and a PA guard column (50 × 4 mm) were used.

The eluents A and B were 150 m*M* sodium hydroxide solution and 150 m*M* sodium hydroxide solution containing 500 m*M* sodium acetate, respectively, and the flow rate was 1 ml/min. The eluents were prepared daily and degassed by a Dionex degas module with helium gas.

Water was purified in 18 MΩ cm by distillation followed by a Milli-Q Labo (Millipore) filtration. A sample solution was made by dissolving the lyophilized material (3 mg) in 1*M* sodium hydroxide (200 μl) and diluting it to 1.0 ml with water. The solution (25 μl) was filtered through a 0.45-μm membrane (Millipore HAWPO1300) and injected into the system. The composition of the eluents was changed as follows: the percentage of eluent B was 40 at 0 min, 50 at 10 min, 60 at 25 min, 70 at 40 min, and 80 at 70 min. All procedures and operations were done at room temperature.

Physical Analyses

X-ray diffraction was performed on wet specimen with a Rotaflex RV-20013 (Rigaku-Denki Co., Tokyo, Japan) under the conditions described by Hizukuri et al (1988). Scanning electron micrographs were taken with a Hitachi model X-610 operated at 10 kV with a beam current of 2×10^{-8} A. Photopastegraphy was performed on a suspension of 0.2% for lotus and corn starches and 0.3% for potato starch at a wavelength of 372 nm; the temperature was raised at 2°C/min (Hizukuri et al 1988).

Amylograms were obtained with a Brabender amylograph (type VA1, 700 cm-g) with 300 g of starch suspension. We used this smaller than normal amount because of the limited amount of the specimen. The temperature was raised at 1.5°C/min. Cold water (8–10°C) was circulated throughout the period, and the heater cover was cooled with a wet cloth during cooling.

RESULTS AND DISCUSSION

General Properties of Lotus Starch

The amylose content of lotus starch calculated from the iodine affinities of starch, amylose, and amylopectin (15.9%) was slightly lower than that calculated from blue values (17.4%) (Table I). These values were similar to that calculated from the recovery of both components in the fractionation (15.7%). Small discrepancies in these calculations are occasionally noted because the chain length dependencies of iodine affinity and blue value are not identical, and the contributions of minor, unidentified materials lost in the fractionation are not accounted for. Both values for the amylose content are lower than those for other tuber and rhizome starches previously studied, including potato (20%), kuzu (20%) (Suzuki et al 1981), and nagaimo, a kind of yam (22–24%) (Suzuki et al 1986), and are similar to that for tapioca (16.7%) (Suzuki et al 1985). However, Sugimoto et al (1984) and Fujimoto et al (1988) reported considerably higher values of 21% from iodine affinity and 21.5–24.5% for three specimens from blue value, respectively. The differences between their values and ours seem to stem mainly from the different assay methods used (they did not measure the iodine affinity or blue value of the amylose and amylopectin of their specimens) and partly from differences in variety and/or growth conditions.

Phosphorus, as phospholipids or phosphate ester at C6 or C3 of a glucosyl residue, is a common minor component of starch. The former is found mainly in cereal starches and the latter in tuber and rhizome starches. Lotus starch contained relatively less phosphorus (Table I) than other tuber and rhizome starches. It was exclusively found in amylopectin, as in the case of other starches, and about half of it was located at C6 of the glucosyl residue. The remainder is speculated to be located at C3, as has been evidenced in potato amylopectin (Tabata and Hizukuri 1971), because phospholipids were completely removed before analysis.

Most of the lotus starch granules were rodlike, measuring 20–40 μm across and 20–90 μm long; small granules were round, with a diameter of 15–25 μm; and some granules were irregular in shape, like potato or sweet potato (Fig. 1). Some granules had dents or hollows at one end. The rough surface of the granules, with fine wrinkles as revealed by high magnification (Fig. 1), appears to be a characteristic property of lotus starch.

The granules gave a Cc-type X-ray powder diffraction pattern (Fig. 2), which is just between A-type and B-type (Hizukuri and Nikuni 1957). Sugimoto et al (1984) and Hizukuri et al (1983, 1985) reported that lotus starches showed the Cb-type and B-type patterns, respectively. These differences suggest that the crystalline structure of lotus starch is easily affected by temperature and some other conditions (Hizukuri et al 1960, 1961, 1980). Fujimoto et al (1988) reported that the crystalline type of their three specimens was Cb, but their X-ray patterns were very similar to that shown in Figure 2. As will be described later, the average chain length of lotus amylopectin was shorter than that of potato amylopectin and longer than that of waxy rice amylopectin. The intermediate crystalline type is in keeping with the relationship between crystalline type and average chain length (Hizukuri et al 1983).

Molecular Structure

The $\overline{DP}n$ and $\overline{DP}w$ values of lotus amylose (Table II) indicate that the molecules of this amylose are among the biggest for amyloses from a variety of sources (Hizukuri 1988). The $\overline{CL}n$ was 540, and the average number of chains was 7.7. The $\overline{CL}n$ value was one of the highest ever reported; others have been in the range of 105–525 (Takeda and Hizukuri 1987, Hizukuri et al 1988, C. Takeda et al 1989). The apparent DP distribution, as defined by the $\overline{DP}w$ of the 10% lowest-weight and highest-weight molecules of the gel-exclusion chromatogram (Fig. 3), was $0.52\text{–}42 \times 10^3$, a considerably wider range than for other sources reported previously, including potato, sweet potato, and tapioca (Hizukuri and Takagi 1984). It was surprising that 10% of the amylose had such a high molecular weight (over 6.5×10^6). The DP at the peak was 6,200.

The plot of log DP versus retention time (Fig. 3) was a concave curve with a steeper slope for the shorter retention times, which

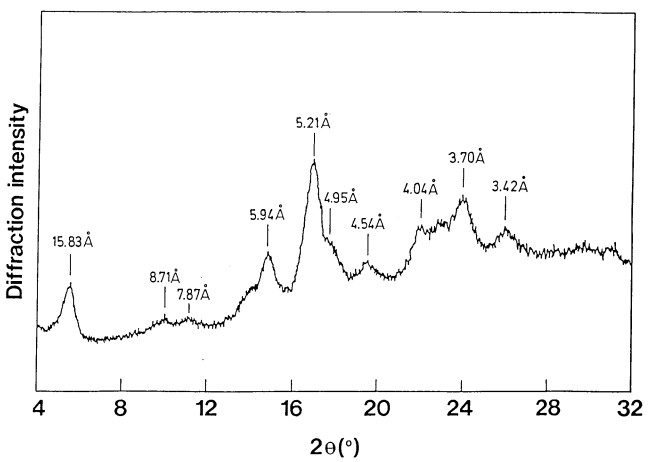

Fig. 2. X-ray diffraction pattern of lotus starch.

TABLE II
Molecular Properties of Lotus Amylose

Property	Value
[η], ml/g	426
$\overline{DP}n$[a]	4,170
$\overline{DP}w$[b]	8,040
$\overline{DP}w/\overline{DP}n$	1.92
Apparent DP distribution	520–42,000
Average chain length	540
Average number of chains	7.7

[a] Number-average degree of polymerization.
[b] Weight-average degree of polymerization.

suggests that lotus amylose may comprise two components, a highly branched, high molecular weight fraction (F_1 in Fig. 3) and a less highly branched, low molecular weight fraction (F_2 in Fig. 3). The weight fraction and $\overline{DP}$ w were 0.43 and 16.2 $\times 10^3$, respectively, for the first fraction and 0.57 and 2.4 $\times 10^3$, respectively, for the second fraction. The high value of the β-amylolysis limit (90%) and the average number of chains of the whole amylose (7.7) suggest that the first and second fractions may be more heavily branched and mainly linear molecules, respectively.

The $\overline{CL}$n values of the amylopectin debranched with isoamylase measured by rapid Smith degradation and by the reducing value were 22.4 and 22.2, respectively (average 22.3). These values were slightly larger than that obtained in a previous study (21.6), probably because in the previous study (Hizukuri et al 1981), the long-chain fraction was removed by precipitation with 1-butanol.

The chain length distribution of debranched amylopectin as revealed by HPLC-LALLS is shown in Figure 4 and Table III. Three peaks at DP 14, 42, and 1,900 and a shoulder at about DP 63 were observed. Some amylopectins have shown two peaks between DP 10 and 20 (Hizukuri 1986), but only one peak appeared in this lotus amylopectin. This kind of profile has been observed also in nagaimo (Suzuki et al 1986) and some cereal amylopectins (Takeda et al 1987). The $\overline{CL}$w of the combined fractions except the long-chain fraction was 25.5. The chain length between two neighboring clusters would be 29, from the difference in average chain length between fractions B_3 and B_2 (Hizukuri 1986). The minute amount of long-chain fraction (1.8%) might be the remains of amylose in the fractionation, but we believe that it is a component of the amylopectin, as in rice amylopectin (Takeda et al 1987). The fraction had considerable effects on some functional properties, such as viscosity, retrogradation tendency (Hizukuri et al 1989), and possibly inclusion capacity of hydrophobic materials. The contribution of this fraction to the functional properties of amylopectin is an interesting problem for future studies.

HPAEC is useful for characterizing the chain length distribution of amylopectin (Koizumi et al 1989, 1991). The chains of lotus amylopectin up to DP 60 were separated into individual peaks (Fig. 5). The DP 6–8 peaks were identified by the authentic specimens, and the other peaks for longer retention times were assumed to be higher homologues. The smallest chain was DP 6, as observed in other amylopectins (Koizumi et al 1991). It was of interest from the structural and biosynthesis perspectives that the DP 8 chain was less than the DP 7 and 9 chains.

The shape of the whole distribution profile differed appreciably from that of the gel-exclusion chromatogram (Fig. 4), which showed much higher response toward the high molecular weight side. This appears to be the result of the continuous decrease in response of the PAD with increasing molecular weight. According to Koizumi et al (1991), the relative PAD response per HCOH or weight of each component over DP 17 decreases gradually as molecular weight increases. The chain length distribution profile, which was corrected for the peak area of the individual component (Fig. 5) by the relative molar PAD response (Koizumi et al 1991), revealed that the most and the least abundant chains between DP 6 and 17 were DP 13 and 8, respectively (Fig. 6). The chain length distribution of this range is characteristic of the species (Koizumi et al 1991).

The response of a differential refractometer (refractive index, RI) to about DP>6 is considered to be constant as to the concentration by weight basis. However, it is apparent that the response of a PAD decreases continuously beyond DP 17 because of its lower response relative to that of RI. Therefore, PAD and

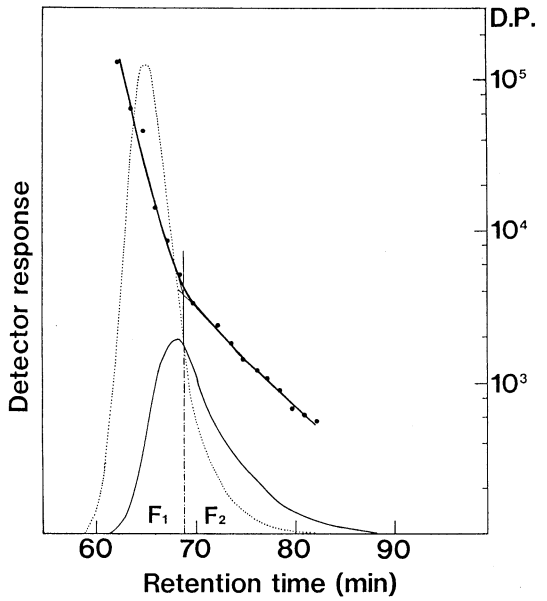

Fig. 3. Gel-exclusion high-performance liquid chromatogram of lotus amylose. Responses of a differential refractometer (—) and a low-angle laser-light-scattering photometer (···). Degrees of polymerization (D.P.) are also plotted (·—·). F_1 and F_2 indicate the two fractions of lotus amylose.

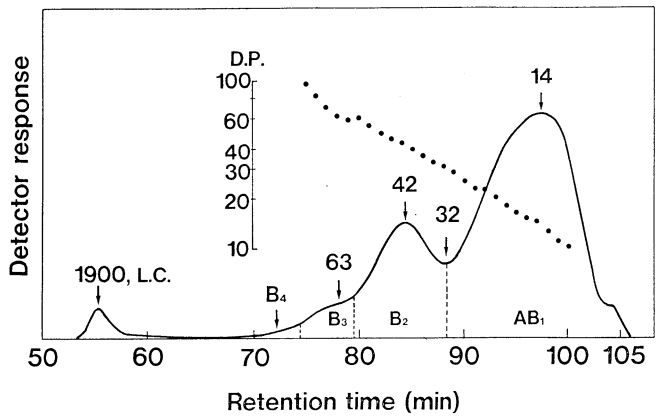

Fig. 4. Gel-exclusion high-performance liquid chromatogram of debranched amylopectin of lotus starch. D.P. = degrees of polymerization; L.C. = long chain; AB_1 and B_2–B_4 are fraction designations.

TABLE III
Chain-Length Distribution of Lotus Amylopectin

	Fraction					
Method	AB_1	B_2	B_3	B_4	Long Chain	AB_1–B_4
$\overline{CL}$w[a]	15.5	43	72	170		25.5
Weight, %	70.7	22.4	4.3	0.8	1.8	
Mole, %	88.6	10.1	1.2	0.1	0	

[a] Weight-average chain length.

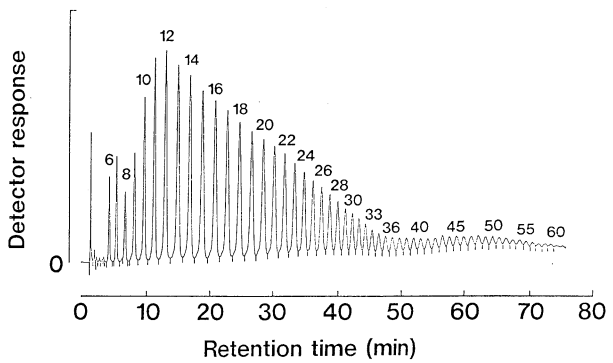

Fig. 5. High-performance anion-exchange chromatogram of debranched amylopectin of lotus starch.

RI detection have advantages for the analysis of low and high molecular weight materials, respectively.

Pasting and Retrogradation

The pasting behavior of lotus starch was compared with that of other starches with the Brabender amylograph. The conditions were chosen to give the same peak viscosity according to Bhattacharya and Sowbhagya (1978). The amylogram of lotus starch at 4.6% (w/w) showed the onset of pasting at 64°C and peak viscosity of 305 BU at 70°C (Fig. 7). The viscosity decreased smoothly to 110 BU after extended heating and cooling. The same level of peak viscosity (300–310 BU) was observed for tapiocas I and II, waxy maize, maize, wheat, sweet potato, and potato starches at concentrations of 6.0, 4.8, 5.6, 6.8, 9.0, 4.8, and 1.5%, respectively. These results imply that lotus starch has an intermediate viscosity like that of tapioca II and sweet potato starches, that is, between those of cereal starches and potato starch. However, the lotus starch showed a considerable breakdown after continuous heating (Table IV). The peak viscosity of the specimen in this study appears to be lower than the 940 BU at 6% and 640 BU at 5% reported by Fujimoto et al (1985), but the large breakdown is common among these specimens. In general, the amylogram of lotus starch resembled those of tapioca II and waxy maize starches, although its pasting temperature was a little lower. The swollen lotus starch granules seemed to be very fragile, were easily broken or deformed during heating with agitation, and showed less gelling tendency upon cooling than other starches tested.

The onset of gelatinization was at 58.5°C on the photopastegram (Fig. 8), which was taken at a somewhat rapid increase in temperature (2°C/min). The paste transparency increased rapidly between 62 and 70°C. This rapid increase was similar to the behavior of potato starch but differs from that of maize starch, in which the controlled increase in transparency after the onset of gelatinization could be due to the presence or formation of amylose-lipid complexes. The large decrease in transparency at the initiation of gelatinization of lotus starch has been observed also in some other starches (Hizukuri et al 1988, Y. Takeda et al 1989), but the reason for the decrease is uncertain.

The pasting properties of lotus starch were also examined by the increase in degree of gelatinization (DG-g and DG-i) of starch suspensions heated at various constant temperatures for 20 min. Gelatinization by these measures started at about 55°C, proceeded rapidly up to 70°C, and was complete at 80°C (Fig. 9). The DG-g values at low temperatures (below 59°C) were higher than the DG-i values, suggesting that the disorganization of the starch granule occurred preferentially in amylopectin. At high temperatures (above 80°C), both DG-g and DG-i values reached 100%. These general behaviors are common to potato and lily starches (Takeda and Hizukuri 1974, Takeda et al 1983) but differ from those of water chestnut (Hizukuri et al 1988), wheat, maize, and rice starches (Takeda and Hizukuri 1974). This may suggest that lotus starch contains as little lipid as potato starch, and in fact only 5 ppm of phosphorus was removed by 85% aqueous methanol extraction (Table I). In general, DG-g is larger than DG-i throughout the pasting process up to 100°C in seed and cereal starches (Takeda and Hizukuri 1974, Hizukuri et al 1988), probably because of the presence of lipids in cereal starches.

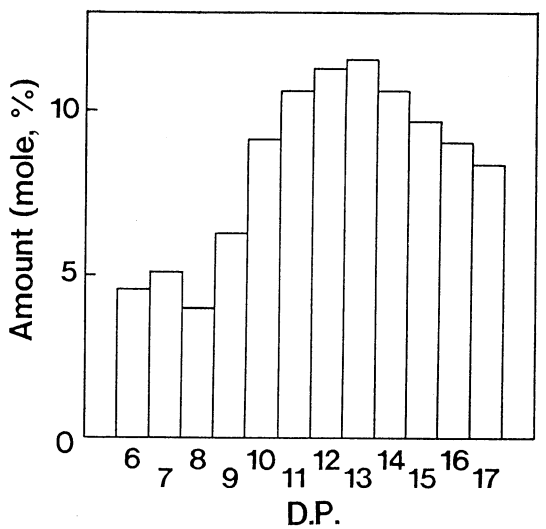

Fig. 6. The molar chain length distribution of lotus amylopectin chains with degree of polymerization (D.P.) 6–17, based on high-performance anion-exchange chromatography (Fig. 5) and corrected by molar responses.

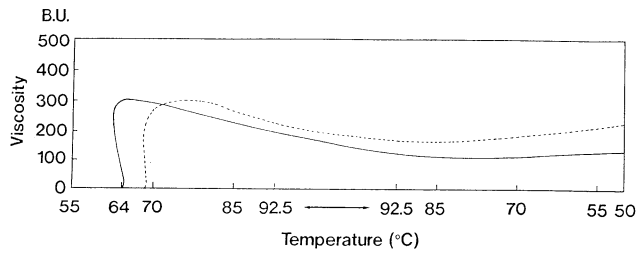

Fig. 7. Amylograms of lotus (4.6%, —) and tapioca II (4.8%, - -) starches.

TABLE IV
Amylogram Characteristics of Lotus and Other Starches

Starch	Concentration (%, w/w)	Pasting Temp. (°C)	Peak Viscosity (BU, °C)	Viscosity at 50°C (BU)	Breakdown[a] (BU)
Lotus	4.6	64	305, 70	135	195
Tapioca I	6.0	65	305, 72	183	165
Tapioca II	4.8	68	298, 82	205	140
Waxy maize	5.6	67	308, 77	180	148
Maize	6.8	70	303, 91	455	80
Wheat	9.0	63	300, 92	413	65
Sweet potato	4.8	73	310, 91	380	8
Potato	1.5	66	300, 92.5	205	110

[a] Peak viscosity minus minimum viscosity after peak viscosity.

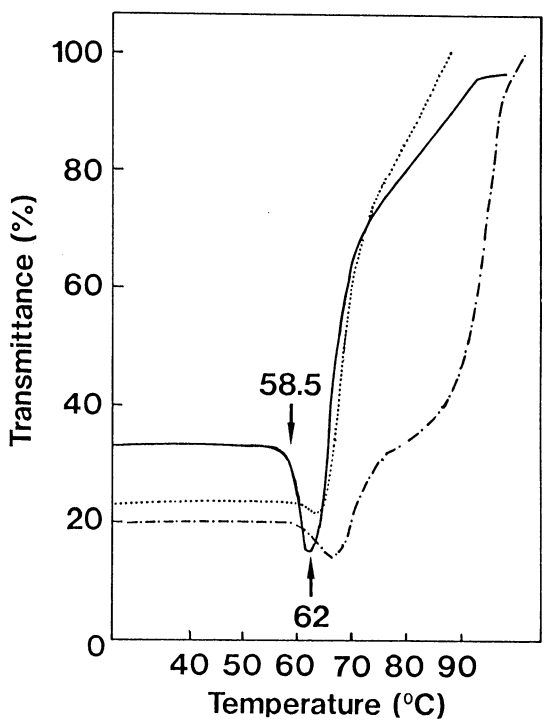

Fig. 8. Photopastegrams of lotus (—), potato (···), and maize (- · -) starches.

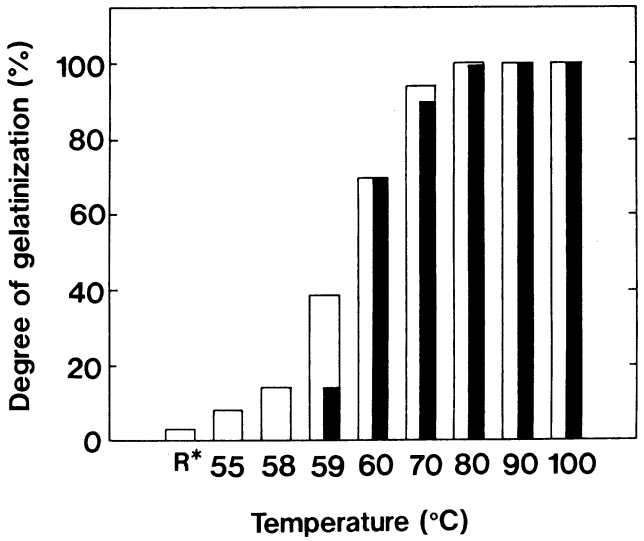

Fig. 9. Temperature dependence of pasting of lotus starch. The degree of gelatinization (DG) was measured by glucoamylase digestion (DG-g, □) and by iodine titration (DG-i, ■). R* indicates raw starch.

TABLE V
Retrogradation of Lotus Starch Paste[a]

Time (hr)	Degree of Gelatinization (DG) (%)	
	DG-g[b]	DG-i[c]
0	100	100
1	100	100
24	99	83
72	96	78
120	82	67

[a] 5% aqueous paste stored at 0° C.
[b] Measured by glucoamylase digestion.
[c] Measured by iodine titration.

The retrogradation tendency of aqueous lotus starch paste was estimated also by DG-g and DG-i on 5% pastes during storage at 0° C (Table V). The paste was stable for 24 hr according to the DG-g value, whereas the DG-i value decreased to 83% at 24 hr and decreased further upon prolonged standing. These data suggest that lotus starch has a slightly faster retrogradation tendency than tapioca starch and retrogrades slower than potato, kuzu (Suzuki et al 1981), lily (Takeda et al 1983), nagaimo (Suzuki et al 1986), and nonwaxy cereal starches (Hizukuri et al 1972). The low increase in amylogram viscosity of lotus starch upon cooling (Fig. 7) also suggests the low retrograding nature of this starch. The low amylose content, large amylose molecules, and relatively short average chain length of the amylopectin, like that of tapioca amylopectin, probably account for the low retrograding nature of lotus starch.

In summary, the characteristics of lotus starch were as follows. Most granules were fairly large and rod-shaped, with very fine wrinkles on the surface. Some granules had dents at one end. Granules exhibited the Cc-type X-ray diffraction pattern. Pasting behavior, as indicated by the Brabender amylograph, resembled that of tapioca and waxy maize starches. The retrogradation tendency of the lotus paste was low. The amylose content was as low as that of tapioca starch. Amylose molecules showed a fairly large DP distribution; some molecules were very large, with DP more than 40,000, and the average chain length was 540, which is the largest ever reported. Lotus amylose may be composed of two components, a highly branched fraction with high DP and a less branched fraction with low DP. The $\overline{CL}$n of lotus amylopectin was intermediate between those of waxy rice and potato starches. The molar chain length distribution between DP 6 and 17 showed a minimum and a maximum at DP 8 and 13, respectively, and the presence of a small amount (1.8%) of long chains. These properties indicate that lotus starch appears to be a suitable material for the production of starch derivatives, rice cakes, and related products.

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