Physicochemical Properties of Lily Starch

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ABSTRACT

The physicochemical properties of starch from lily (Maximovicz's lily, *Lilium maximoroiczii*, Regel) bulbs were investigated in detail. The starch contained 25% amylose (as judged from the iodine affinity) and 60 ppm of phosphorus. The limiting sedimentation coefficient and limiting viscosity number $[\eta]$ of the amylose were 2.33×10^{-13} sec (90% dimethyl sulfoxide at 25° C) and 312 ml/g (1*M* KOH at 22.5° C), respectively. The amylose was found to be a branched molecule of d.p. 2,300 with an average of 4.9 chains by analyses of reducing and nonreducing residues and was hydrolyzed 89% with sweet potato β-amylase. The lily amylopectin was found to have a chain length of d.p. 23.3 by rapid Smith degradation and was hydrolyzed 57% with β-amylase. A clear difference was observed between the chain-length distribution of lily and potato amylopectins on gel-filtration chromatography. The amylograph of lily starch showed a lower maximum viscosity and considerably lower breakdown than did the amylograph of potato starch. Lily amylose showed a higher retrogradation tendency than potato amylose.

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Suzuki et al (1981) reported the physicochemical properties of kuzu starch and suggested that the gelatinization and retrogradation properties are influenced by the structure of amylose. We devised new methods for determining reducing and nonreducing terminal residues of amylose and revealed that amylose samples from several plant sources differ in molecular weight and branched structure (Hizukuri et al 1981). We were interested in the relationship between the functional properties and the molecular structures of starch. In this study, we singled out lily starch and examined the physicochemical properties in detail and compared them with those of potato starch. Lily starch has the same crystalline structure (B-type) as potato starch, but contains an appreciably higher amount of amylose and was found to have some other characteristic properties.

MATERIALS AND METHODS

Materials

Starch was prepared from lily (Maximovicz's lily, *Lilium maximoroiczii*, Regel) bulbs cultivated for food in Hokkaido and obtained from a local market. The bulbs were homogenized in a home blender with cold water, and the homogenate was squeezed through bleached cotton cloth. The liquid slurry was stored in a refrigerator until the starch had settled at the bottom of the beaker. The supernatant liquor was decanted and the tailings layer removed by scraping with a spatula. The prime starch was reslurried in cold water, and the suspension was squeezed again through bleached cotton cloth and stored in a refrigerator. These procedures, except squeezing, were repeated many times until the starch appeared to be pure. The prime starch was dried at room temperature, and the dry specimen (water content 19.1%) was stored in a refrigerator until use. The nitrogen content of lily starch was determined to be 0.006% by a micro-Kjeldahl method (Bailey 1967).

Fractionation of lily starch into amylose and amylopectin was performed by the method of Lansky et al (1949) under an atmosphere of nitrogen to avoid oxidative degradation (Baum and Gilbert 1954, Cowie and Greenwood 1957). Lily amylose was purified by recrystallization six times from hot 10% l-butanol with cooling under a nitrogen atmosphere. The yields of amylose and amylopectin from lily starch (8.7 g dry weight) were 1.9 and 4.5 g, respectively.

Potato amylose and amylopectin were prepared by fractionating a commercial starch (Hokuren, Hokkaido), which was washed several times with distilled water before use. Their properties in part were reported previously (Hizukuri et al 1981; Takeda and Hizukuri 1981, 1982).

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Sweet potato β-amylase was prepared by the method described previously (Takeda and Hizukuri 1969) and recrystallized from ammonium sulfate solution to provide stability during storage. Crystalline *Pseudomonas* isoamylase, purified by the method of Kato et al (1977), was a gift from T. Harada (Osaka University). Pullulanase (*Aerobactor aerogenes*, crystalline) was a product of Hayashibara Biochemical Laboratories Inc. Bio-Gel P-30 and Sephadex G-75 were obtained from Bio-Rad Laboratories and Pharmacia Fine Chemicals, respectively.

Methods

Iodine affinity was determined at 30°C by the amperometric titration procedure of Larson et al (1953). The blue value is twice the absorbance at 680 nm measured in a solution (10 m*M* acetate buffer, pH 4.8) containing polysaccharide (2 mg/100 ml), iodine (4 mg/100 ml), and potassium iodide (40 mg/100 ml) in a 10-mm cell, using a Hitachi 139 spectrophotometer. For these determinations, lily starch was defatted with 80% dioxane for 48 hr using a Soxhlet extraction apparatus.

The amylose content of the starch was calculated from the iodine affinity (IA) and blue value (BV) with the following equation, in which amylose content was equal to:

$$\frac{[\text{IA (BV) of defatted starch}] - [\text{IA (BV) of amylopectin}]}{[\text{IA (BV) of amylose}] - [\text{IA (BV) of amylopectin}]} \times 100.$$

The sedimentation coefficient, s, of amylose dissolved in 90% dimethyl sulfoxide was determined at 25°C with a Hitachi 282 analytical ultracentrifuge. The values of s were determined at four amylose concentrations, c (2, 4, 6, and 8 mg/ml), and the limiting sedimentation coefficient was estimated by extrapolation of $1/s$ vs c to infinite dilution.

The limiting viscosity number $[\eta]$ was determined in 1*M* KOH with an Ostwald viscometer at 22.5° C. The flow time was 128 sec for 1*M* KOH.

An amylogram was obtained using a Brabender amylograph (type DC 3, 700 CM-GRS). The temperature was raised and lowered at a rate of 1.5°C/min. The maximum temperature of 92.5°C was maintained for 15 min. The cup was rotated at 75 rpm.

X-ray diffraction was performed under the conditions described elsewhere (Hizukuri et al 1980).

Phosphorus was determined as inorganic phosphate by the method of Itaya and Ui (1966) after treatment with hot perchloric acid (Allen 1940). The phosphate group bound to C-6 of the glucosyl residue was assayed by the method described previously (Hizukuri et al 1970).

β-Amylolysis was performed by incubating 200 μg of polysaccharide in 0.2 ml of 20 m*M* acetate buffer, pH 5.5, with 50 U (μmole maltose released per minute) of β-amylase at 37°C. The simultaneous digestion of amylose with β-amylase and pullulanase was done by adding 0.8 U of pullulanase to the above mixture and incubating at 37°C for 20 hr. Reducing sugar produced was

determined by the methods of Somogyi (1952) and Nelson (1944).

The average chain length of amylopectin was determined by two methods: rapid Smith degradation (Hizukuri and Osaki (1978) and hydrolysis with isoamylase (Suzuki et al 1981). The average degree of polymerization ($\overline{d.p.}$) and average chain length of amylose were measured by the modified Park-Johnson and rapid Smith degradation methods (Hizukuri et al 1981), respectively. The average chain number of amylose was the value of $\overline{d.p.}$ divided by average chain length.

The distribution of chain lengths of amylopectin debranched with isoamylase was examined by gel filtration on Bio-Gel P-30 or Sephadex G-75 under the conditions described elsewhere (Suzuki et al 1981).

The degree of gelatinization (DG) of a paste was determined by glucoamylase digestion (DG-g) and iodine titration (DG-i) (Suzuki and Hizukuri 1979).

RESULTS AND DISCUSSION

The properties of Maximovicz's lily starch and its amylose and amylopectin components are summarized in Table I.

Amylose Content

The iodine affinity and blue value of lily amylose were 20.0 and 1.49, respectively. The amylose content of the starch was calculated to be 25.0 and 26.8% from the iodine affinity and blue value, respectively. These values are the lowest compared with the values reported previously. Taki (1959) reported that the amylose content of lily starch (no description of the origin) was found to be 28 and 31% on iodine titration and fractionation of amylose by paper chromatography, respectively (Taki 1959, 1962). Easter lily starch contained 33% (Fujimoto et al 1981) and 34% (Bates et al 1943) amylose. Although the amylose content may vary to some extent with the determination method, lily starch appears to contain a higher amount of amylose than other starches of roots such as potato (20–23%, Anderson and Greenwood 1955, Greenwood and Thomson 1962), tapioca (16.9%, Ohashi 1956), kuzu (20.4%, Suzuki et al 1981), arrowroot (20.5% Anderson et al 1955), taro (12%, Fujii et al 1961; 14% Higashihara et al 1975), and sweet potato (17–22%, Doremus et al 1951, Ohashi 1957).

Structure of Amylose

Lily amylose contained 2 ppm of organic phosphorus, which was lower than the values for potato and kuzu amyloses (Suzuki et al 1981). The sedimentation pattern of lily amylose (Fig. 1a) was somewhat asymmetrical and was similar to that of potato amylose (Fig. 1b). The limiting sedimentation coefficients for lily and potato amyloses in 90% dimethyl sulfoxide at 25°C were 2.33×10^{-13} sec and 2.76×10^{-13} sec, respectively. The limiting viscosity number $[\eta]$ of lily amylose in 1 *M* KOH at 22.5°C was 312 ml/g. This value was between those of kuzu (228 ml/g, Suzuki et al 1981) and potato (470 and 520 ml/g, Cowie and Greenwood 1957; 420 ml/g, Suzuki et al 1981) amyloses. These results indicate that the molecular weight of lily amylose is appreciably smaller than that of potato amylose, which was confirmed by determination of the reducing residue. The $\overline{d.p.}$ of lily amylose was determined to be 2,300 by a modified Park-Johnson method (Hizukuri et al 1981). The molecular weight of lily amylose was between those of kuzu ($\overline{d.p.}$ 1,590) and potato ($\overline{d.p.}$ 4,850 and 6,340, Hizukuri et al 1981) amyloses. The hydrolysis of lily amylose with β-amylase reached a limit value of 89% in 30-min incubation, and this was maintained for 6 hr. The digestion mixture remained very clear and retained almost full β-amylase activity during the incubation. The addition of pullulanase to the digest (without inactivation of β-amylase) after 3-hr incubation with β-amylase gave complete conversion to maltose as judged by the methods of Somogyi (1952) and Nelson (1944). However, the digest was stained faint blue on the addition of iodine solution, suggesting that a tiny amount (probably less than 0.5%) of amylose still remained in the digest, probably due to the presence of a very small amount (2 ppm) of bound phosphate in the amylose since β-amylase is unable to bypass the phosphorylated residue (Takeda and Hizukuri 1981). A similar observation was reported for potato amylose when it was hydrolyzed with glucoamylase (Abe et al 1982). These findings indicate that the incomplete hydrolysis of lily amylose with β-amylase is not due to retrogradation but to the presence of the branch linkage. In fact, lily amylose was found to be composed of an average of 4.9 chains with an average chain length

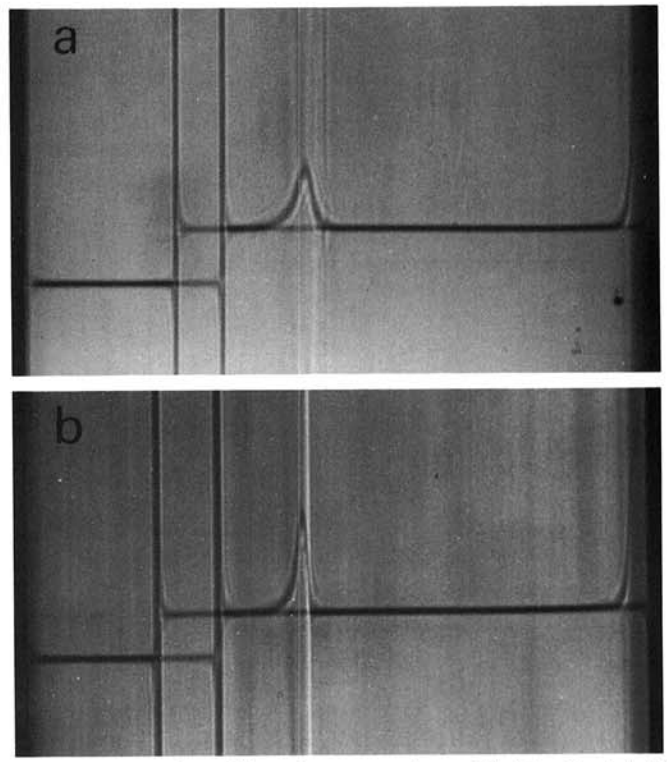

Fig. 1. Sedimentation of lily and potato amyloses. Lily **(a)** and potato **(b)** amyloses were sedimented at 57,000 rpm at 25°C with an ultracentrifuge. Photographs were taken 2 hr after the maximum speed was reached. The concentration of amylose was 4 mg/ml of 90% dimethyl sulfoxide. The degree of polymerization of potato amylose was 6,340.

TABLE I
Properties of Lily Starch and Its Components

Components	Iodine affinity I_2 mg/100 mg	Blue Value	Amylose Content (%)	β-Limit (%)	$\overline{d.p.}$[a]	Chain length (d.p.) Smith Degradation	Chain length (d.p.) Isoamylase	Chain Number	P content[b] (ppm) Po	P content[b] (ppm) P-6
Starch, defatted	5.27	0.518	25.0[c] 26.8[d]	64		34.4	33.5		60	33
Amylopectin	0.37	0.163		57		23.3	23.9		75	42
Amylose	20.0	1.49		89	2,300	474		4.9	2	

[a] Degree of polymerization.
[b] Po = organic phosphorus, P-6 = phosphorus bound at C-6 of the glucosyl residue.
[c] From iodine affinity.
[d] From blue value.

of $\overline{d.p.}$ 474 by analyses of nonreducing and reducing residues. The average chain number of lily amylose was lower than those of potato, kuzu, and tapioca amyloses, and the average chain length of lily amylose was a little shorter than that of potato amylose (Hizukuri et al 1981). From these results, we concluded that lily amylose is a smaller molecule with slightly shorter chains than potato amylose.

Structure of Amylopectin

The average chain length of lily amylopectin was determined to be $\overline{d.p.}$ 23.3 and 23.9 by rapid Smith degradation and by debranching with isoamylase, respectively. These values agree well with that for potato amylopectin ($\overline{d.p.}$ 23.7) but are higher than that for kuzu amylopectin ($\overline{d.p.}$ 20.5, Suzuki et al 1981). Lily starch and amylopectin contained 66 and 75 ppm of organic phosphorus, respectively. The phosphorus content of lily amylopectin was approximately one eighth that of potato amylopectin (604 ppm) and half that of kuzu (158 ppm, Suzuki et al 1981) and sweet potato amylopectins (135–204 ppm, Tabata and Hizukuri 1975). Fifty-six percent of the phosphorus of lily amylopectin was bound to C-6 of the glucosyl residue, and the remainder is supposed to bind to C-3 as reported for potato amylopectin (Hizukuri et al 1970). The β-amylolysis limit (57%) of lily amylopectin was the same as those of potato and kuzu amylopectins (Suzuki et al 1981). These results suggest that the rough structures of lily and potato amylopectins resemble each other except for the amount of esterified phosphate. However, the detailed structures in the distribution of chain lengths differ from each other as described below.

Lily amylopectin was debranched with isoamylase, and the resulting chains were fractionated by gel filtration on Bio-Gel P-30, and the distribution of the chains was compared with that for potato amylopectin (Fig. 2). The elution curve of debranched lily amylopectin was similar in outline to that of debranched potato amylopectin but differed clearly in the carbohydrate distribution and $\overline{d.p.}$ of peak fractions. The chains of both debranched amylopectins were fractionated into four fractions, F1, F2, F3, and F4, in order of elution, as seen in Fig. 2. The percentages (mean ± standard deviation) of the carbohydrate amounts of F1, F2, F3, and F4 were 3.4 ± 0.5, 9.3 ± 0.5, 26.2 ± 0.6, and 61.5 ± 1.5% for lily amylopectin (three experiments) and 14.7 ± 0.4, 8.5 ± 0.5, 21.3 ± 0.5, and 55.4 ± 0.5% for potato amylopectin (three experiments), respectively. The carbohydrate amount of lily F1 was smaller than that of potato F1. The organic phosphorus (98%) of both amylopectins was recovered in F1. Lily and potato F1 contained one phosphate group per 80 and 42 glucosyl residues, respectively. The $\overline{d.p.}$ per reducing and nonreducing terminals of potato F1 had the same value of 41. These properties of potato F1 agreed well with those of the phosphorylated chain obtained by ion-exchange chromatography of debranched potato amylopectin with isoamylase (Takeda and Hizukuri 1981). Therefore, F1 is composed of linear molecules carrying a phosphate group; incompletely debranched material, as suggested by Gunja-Smith et al (1970) and Lii and Lineback (1977), is only minor if present. Thus, the carbohydrate amount of F1 depends on the phosphorus content of amylopectin. The phosphorylated chain moved much faster than the corresponding neutral chain of a similar $\overline{d.p.}$ under the conditions of elution with distilled water. This behavior was also observed when Sephadex G-75 was used instead of Bio-Gel P-30. The movement of the faster phosphorylated molecules may be due to the repulsion between intermolecular mutual molecules and the resin and the molecules. Sephadex gel contains a very small amount of carboxyl group.

The carbohydrate amount and $\overline{d.p.}$ of the shoulder fraction of lily F2 were similar to those of potato F2. The $\overline{d.p.}$ of the peak fraction of lily F3 was higher than that of potato F3 and close to that of the kuzu fraction corresponding to F3 ($\overline{d.p.}$ 51, Suzuki et al 1981). The carbohydrate amount of lily F4 was higher than that of potato F4, but both F4s had the same $\overline{d.p.}$ value at the peak fraction.

Possibly, F2 and F3 originate from long and short B-chains (the chain with one or more side chains), respectively, whereas F4 originates mainly from the A-chain (the chain without side chains) as suggested by Akai et al (1971). It may be concluded that lily amylopectin has more A-chains, slightly longer B-chains, and a

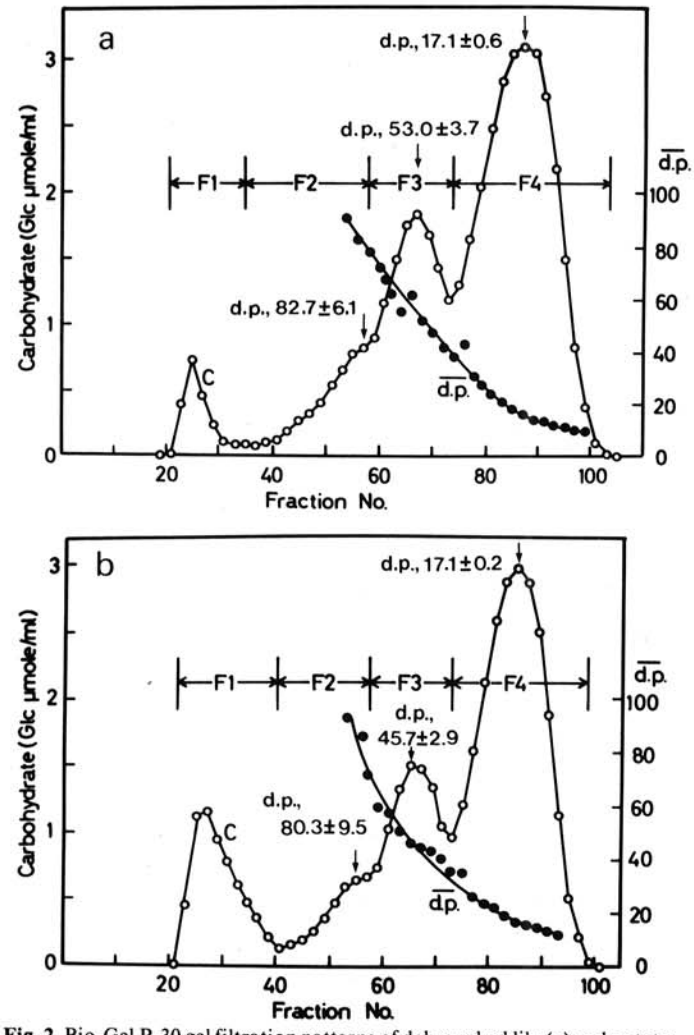

Fig. 2. Bio-Gel P-30 gel filtration patterns of debranched lily **(a)** and potato **(b)** amylopectins. Potato amylopectin used in this experiment contained 604 ppm of organic phosphorus, and had an average chain length of degree of polymerization 23.7 and a β-amylolysis-limit value of 57%. The conditions of gel filtration were described previously (Suzuki et al 1981). The degree of polymerization of peak and shoulder fractions are indicated as means ± standard deviation (three experiments). o = carbohydrate, • = degree of polymerization.

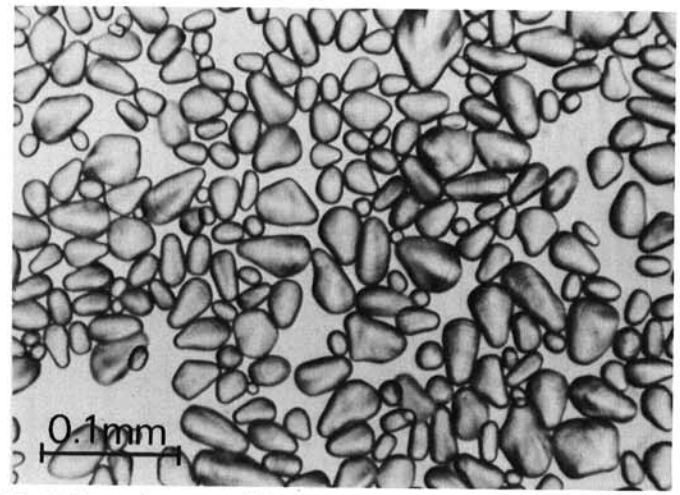

Fig. 3. Photomicrogram of lily starch.

much smaller amount of phosphorylated chains than potato amylopectin.

Structure of Granules

The granules of lily starch were slenderly ovoid, elliptical, and polygonal, and a layered structure was observed in large granules (Fig. 3). The average granule size was 18 and 30 μm for the minor and major axes, respectively. The X-ray diffraction pattern of lily starch was the typical B-type, the same as for potato starch.

Pasting Properties

The profile of an amylogram of lily starch (5%) is shown in Fig. 4. The pasting temperature and maximum viscosity were 62.5°C and 430 Brabender units (BU), respectively. Slight breakdown occurred on keeping the paste at 92.5°C, but not as large a breakdown as in potato starch. This profile was similar to that of sweet potato starch. Pasting of lily starch in 5% 3-methyl-l-butanol caused a considerable decrease in the viscosity and a rise in the pasting temperature. A similar effect of 3-methyl-l-butanol on potato starch was observed (Fig. 4), although Gerlsma (1970) reported that monohydric alcohols such as ethanol, propanol, and butanol lowered the gelatinization temperature. This response to 3-methyl-l-butanol differed from those of cereal, sweet potato, and kuzu starches; that is, 3-methyl-l-butanol lowered the pasting temperature of these starches and raised the viscosity of cereal starches, and barely influenced the viscosity of sweet potato and kuzu starches (Hizukuri and Takeda 1978, Suzuki et al 1981).

Suspensions of lily and potato starches were kept at constant temperatures (50–100°C) for 20 min as indicated in Fig. 5, and the pasting was monitored by the glucoamylase-digestion (DG-g) and iodine titration (DG-i) methods. The onset of the pasting of lily and potato starches was at 55 and 58°C, respectively, with both methods. Such a slight difference in the pasting temperature may not be of importance for characterizing starches of different botanical origins, since the pasting temperature depends on the temperature of growth of the plant (Hizukuri 1969). Amylose appears to control the gelatinization below 70°C in lily starch similarly as in potato starch since DG-g was higher than DG-i. This pasting property is similar to those of wheat, corn, and sweet potato starches (Takeda and Hizukuri 1974), but differs from that of kuzu starch since kuzu amylose and amylopectin were pasted simultaneously (Suzuki et al 1981). Above 80°C, lily starch was fully pasted as indicated by DG-g and DG-i. In this property it differs from wheat and corn starches of which the DG-i remains at about 80% at 100°C (Takeda and Hizukuri 1974). Possibly this is due to the presence of a stable lipid-amylose complex in these cereal starches not found in lily starch.

Retrogradation

The retrogradation property of lily starch paste stored at 0°C was examined by monitoring the DG-g and DG-i values (Table II). The DG-i value decreased rapidly within the first hour and then slowly while the DG-g value decreased gradually during storage, indicating that preferential retrogradation occurs in amylose (Hizukuri et al 1972). The retrogradation tendency of lily amylose was similar to that of kuzu amylose and appreciably higher than that of potato amylose (Suzuki et al 1981). The slightly higher amylose content of lily starch may be in part responsible for the rapid progress of retrogradation. In addition, these results may support the suggestion made by Whistler (1953), that potato amylose of high molecular weight retrogrades slowly because of a steric effect, since lily and kuzu amyloses are considerably smaller molecules than potato amylose. On the other hand, lily amylopectin retrograded more slowly than potato amylopectin, as judged from the DG-g value during storage, but had a retrogradation tendency similar to that of kuzu amylopectin (Suzuki et al 1981). The reason that lily amylopectin, which binds less phosphate, shows a lower retrogradation tendency than potato amylopectin is not presently understood.

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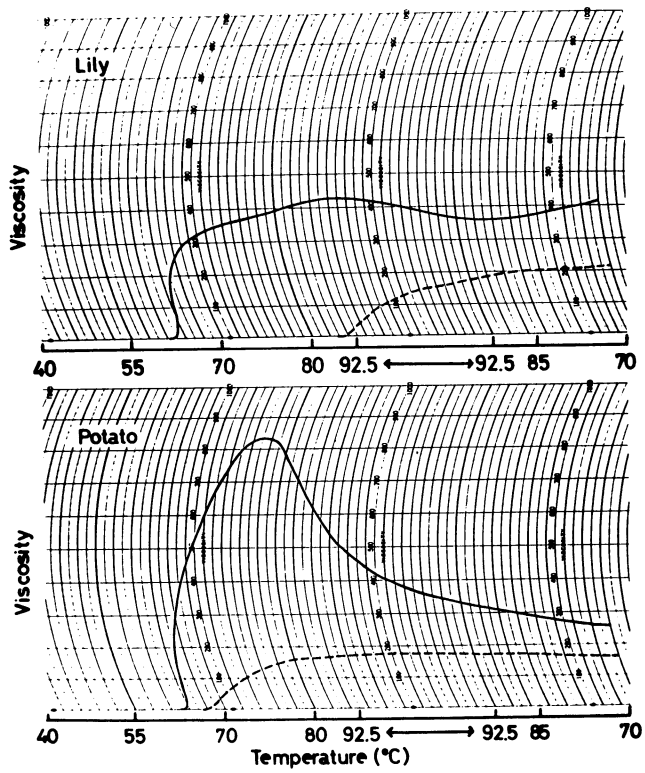

Fig. 4. Brabender amylograms of lily (5%) and potato (4%) starches. Pasting with water (———) and 5% 3-methyl-1-butanol (-----).

TABLE II
Retrogradation Tendency of Lily Starch Paste[a] Stored at 0°C

Storage Time (hr)	DG-g[b] (%)	DG-i[c] (%)
0	100	100
1	95	48
15	93	42
24	89	40
72	84	39

[a] Each paste was prepared by heating 5% starch suspension at 100°C for 20 min as described previously (Suzuki et al 1981).
[b] Degree of gelatinization measured by glucoamylase digestion.
[c] Degree of gelatinization measured by iodine titration.

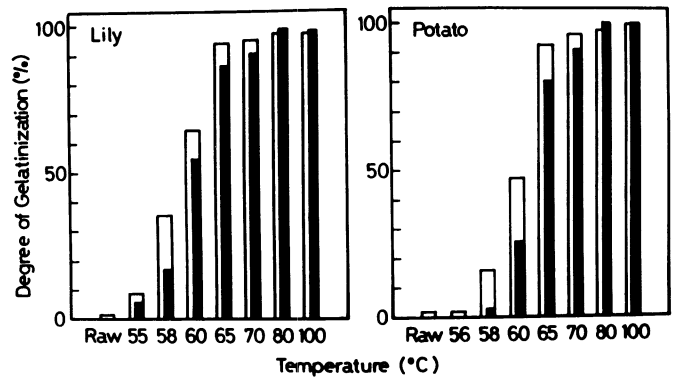

Fig. 5. Temperature dependence of pasting of lily and potato starches. Degree of gelatinization was measured by the glucoamylase-digestion (□) and iodine-titration (■) methods.

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