Physicochemical Studies of Kuzu Starch

A. SUZUKI,[1] S. HIZUKURI,[2] and Y. TAKEDA[2]

ABSTRACT

The physicochemical properties of the starch of kuzu (a wild vine, *Pueraria hirsuta* Matsum) were studied. The starch contains 21% amylose and 124 ppm of phosphorus, which was almost exclusively in the amylopectin. The β-amylolysis limits of the amylose and amylopectin were 76 and 57%, respectively. The average chain length of the amylopectin was 20.5, which was shorter than that (22.0) of potato amylopectin. The limiting viscosity numbers of the amylose and amylopectin were 228 and 160 ml/g (in 1 *M* KOH at 22.5° C), respectively. These values were considerably lower than those of potato amylose and amylopectin. The amylograph (6%) of the starch showed a low maximim viscosity (245 BU), and the viscosity was fairly stable during heating and cooling. Kuzu's amylopectin may be more resistant but its amylose less resistant to retrogradation than are those of potato starch. Granules are of the C_a crystalline type, similar to those of sweet potato starch.

Cereal Chem. 58(4):286–290

Kuzu (*Pueraria hirsuta* Matsum) is a wild vine that grows in central and southern Japan. This deciduous plant stores starch in the roots in winter. The yield of starch is about 15% of the fresh roots. Although production is only about 300 tons per year, the starch is highly prized in traditional confectionery, the manufacture of a kind of noodle, and cooking. It has been the subject of only limited studies (Aoki and Tani 1975, Suzuki et al 1958) and has not been well characterized. The purpose of this study was to examine in detail the structure and properties of the starch.

MATERIALS AND METHODS

Materials

Kuzu and potato starch used in this study were produced in the factories of Hirohachido-shoten (Kagoshima) and Hokuren (Hokkaido), respectively, by similar processes. The starches were washed several times with distilled water and defatted by extraction five times with 85% aqueous methanol for 4 hr under refluxing (Schoch 1942). The nitrogen content of the kuzu starch was 0.016%. Fractionation of the starch into amylose and amylopectin was done by the method of Lansky et al (1949) under a nitrogen atmosphere to avoid oxidative degradation (Baum and Gilbert 1954, Cowie and Greenwood 1957b). The amylose was recrystallized four times from hot 10% *n*-butanol by cooling under nitrogen. The yields of amylose and amylopectin from the kuzu starch were 17.5 and 67%, respectively.

β-Amylase was prepared by the method described previously (Takeda and Hizukuri 1969) and recrystallized from aqueous ammonium sulfate to provide stability during storage.

Crystalline isoamylase (*Pseudomonas*), purified by the method of Kato et al (1977) was a gift from T. Harada (Osaka University).

Chemicals were of the special grade of Wako Pure Chemical Industries, Ltd., Osaka.

Methods

Iodine affinity was determined at 30° C by the amperometric titration procedure of Larson et al (1953).

The absorption spectrum of the glucan-iodine complex was

[1]Laboratory of Food Science, Osaka Women's University, Daisen-cho, Sakai 590, Japan.

[2]Department of Agricultural Chemistry, Faculty of Agriculture, Kagoshima University, Korimoto, Kagoshima 890, Japan.

0009-0352/81/04028605/$03.00/0

TABLE I
Properties of Kuzu Starch

Components	Iodine Affinity (I_2, mg/100 mg)	Blue Value	Amylose Content (%)	β-Limit (%)	Chain Length ($\overline{dp}$[a])	P Content[b] (ppm)	
						Po	P6
Starch	4.06	0.406	20.8,[c] 21.0[d]	65	26.5[e]	124	···
Amylopectin	0	0.135	···	57	20.4,[e] 20.5[f]	158	121
Amylose	19.5	1.41	···	76	···	10	···

[a] Degree of polymerization.
[b] Po = organic phosphorus, P6 = phosphorus bound at C-6 of glucosyl residues.
[c] From iodine affinity.
[d] From blue value.
[e] By rapid Smith degradation.
[f] By degradation with isoamylase.

measured in a solution (8m*M* acetate buffer, pH 5.0, 100 ml) containing 4 mg of glucan, 8 mg of iodine, and 80 mg of potassium iodide in a 10-mm cell, using a Hitachi recording spectrophotometer. Blue value is defined as the absorbance at 680 nm measured under the above conditions. Amylose content was calculated from the iodine affinity and/or blue value.

Nitrogen content was determined by a micro-Kjeldahl method (Bailey 1967).

Phosphorus content was determined as inorganic phosphate by the method of Fiske and Subbarow (1925) after treatment with hot perchloric acid (Allen 1940). Ester phosphate positioned at C-6 of the glucose residues was estimated by the method described previously (Hizukuri et al 1970).

β-Amylolysis was performed at 30° C in 50m*M* acetate buffer (3 ml), pH 4.8, containing 3 mg of starch or its components and 150 μmole/min of sweet potato β-amylase. The hydrolysis reached a maximum value after 20 min of incubation and maintained it for 6 hr.

Isoamylolysis was conducted for 3 hr at 50° C in 22.7m*M* acetate buffer (2.75 ml), pH 3.5, containing 25 mg of amylopectin and 2.1 μmole/min of crystalline *Pseudomonas* isoamylase. The reducing value due to the hydrolysis was constant in the incubation for 0.5–5.0 hr. The reducing value equivalent to that of glucose was determined by Somogyi-Nelson's method (Nelson 1944, Somogyi 1952), but heating was with Somogyi reagent for 30 min as described elsewhere (Hizukuri et al 1970).

The average chain length of amylopectin was determined by rapid Smith degradation (Hizukuri and Osaki 1978) and hydrolysis with isoamylase. The latter was done under the described conditions and degree of polymerization ($\overline{dp}$) was calculated from the reducing value equivalent to glucose and total carbohydrate measured by the Anthrone-H_2SO_4 method (Koehler 1952).

The distribution of the chain length of debranched amylopectin was examined by gel-filtration. Immediately after the isoamylolysis, the reaction mixture (7 mg/ml) was applied to a column (2.6 × 100 cm) packed with Bio-Gel P-30 and eluted upwards with distilled water. The flow rate was maintained at 40 ml/hr and fractions of 5 ml were collected. The column was maintained at 50 ± 0.5° C by circulating water.

The degree of gelatinization (DG) was estimated by glucoamylase digestion (DG-g) and iodine titration (DG-i) as described previously (Suzuki and Hizukuri 1979). One gram of starch was suspended in 19 ml of water, and the suspension was heated in a water bath at several temperatures for 20 min with gentle stirring. The DG-i value is specific for amylose.

The limiting viscosity number [η] was measured in 1*M* KOH, using an Ostwald viscometer at 22.5° C. Dry specimen was dissolved in 1*M* KOH at room temperature in a Potter homogenizer.

Amylograms were taken with a Brabender amylograph (Type DC 3, 700 CM/GRS). The temperature was raised or lowered at 1.5° C per min. The maximum temperature of 92.5° C was maintained for 15 min.

X-ray diffraction was conducted with an X-ray diffractometer (Rigakudenki Model 17-3P). The operation conditions were as described elsewhere (Hizukuri et al 1980).

TABLE II
Characteristics of Iodine-Stained Potato and Kuzu Starches

Starch Components	Potato			Kuzu		
	λ_{max}[a] (nm)	Absorbance at		λ_{max} (nm)	Absorbance at	
		λ_{max}	680 nm		λ_{max}	680 nm
Amylose	656	1.56	1.49	656	1.43	1.41
Amylopectin	560	0.350	0.193	556	0.240	0.135
Starch	598	0.545	0.473	616	0.477	0.406

[a] Maximum absorption wavelength.

RESULTS AND DISCUSSION

The general properties of kuzu starch and its components are summarized in Table I.

Structure of Amylose

The amylose content of kuzu starch was determined to be 20.8 and 21% from the iodine affinity and blue value, respectively. These values agreed well with that (20.2%) reported by Aoki et al (1975). The iodine affinity (19.5) and β-amylolysis limit (76%) of kuzu amylose imply that it has similar properties to that from other sources (Banks and Greenwood 1975). The incomplete hydrolysis with β-amylase suggests the presence of a few branch linkages in the amylose (Banks and Greenwood 1966, 1967; Kjølberg and Manners 1963) and studies on the branched structure are under way. The starch contained 124 ppm of organic phosphorus but the amylose contained only 10 ppm. The light absorption spectra of iodine-stained solutions of kuzu and potato amyloses were almost identical (Table II). The [η] of kuzu amylose in 1*M* KOH at 22.5° C was 228 ml/g (Fig. 1). This value corresponds to 1,700 $\overline{dp}$ according to the formula: $\overline{dp} = 7.4[\eta]$ (Cowie and Greenwood 1957a, Greenwood 1970). The [η] of kuzu amylose appears to be about half the size of that of potato amylose, which is [η] = 435 ml/g.

Structure of Amylopectin

The iodine-amylopectin spectrum of kuzu differed considerably from that of potato amylopectin (Table II). The lower λ_{max} and absorbance at λ_{max} and 680 nm of kuzu amylopectin compared to the values for potato amylopectin suggest that the chains of the former are shorter than those of the latter. This was confirmed by the determination of chain length; that is, $\overline{dp}$ of kuzu and potato amylopectins were 20.5 and 22.0, respectively. The phosphorus content of kuzu amylopectin was 158 ppm, which was approximately one fourth that of potato amylopectin (604 ppm). Most of the phosphorus (77%) was bound at C-6, and the rest of the phosphorus was assumed (it was not examined) to be located mainly at C-3 as reported in potato amylopectin (Tabata and Hizukuri 1971). The [η] of kuzu amylopectin (160 ml/g, in 1*M* KOH at 22.5° C) was lower than that of potato amylopectin (224 ml/g) as shown in Fig. 1. This suggests a lower molecular weight of kuzu amylopectin or a more spherical molecule with a more compact structure.

To characterize the distribution of chain length of the amylopectin, it was debranched completely with *Pseudomonas* isoamylase and was subjected to gel-permeation chromatography.

The resulting elution pattern showed that the chains were composed of three fractions, F_1, F_2, and F_3, in order of elution (Fig. 2). The $\overline{dp}$ of F_1 was 91 and the dp of the peak fractions of F_2 and F_3 were 51 and 16, respectively. F_1, occasionally observed by other workers, has been considered to be an incompletely debranched material (Gunja-Smith et al 1972, Lii and Lineback 1977). In the present experiment, F_1 contained 44% of the phosphate ester in the original amylopectin. This fraction may contain a small amount of undebranched material but we think it is mainly composed of linear phosphorylated molecules. Phosphorylated chains prepared by debranching potato amylopectin are essentially linear $\overline{dp}$ = 42,

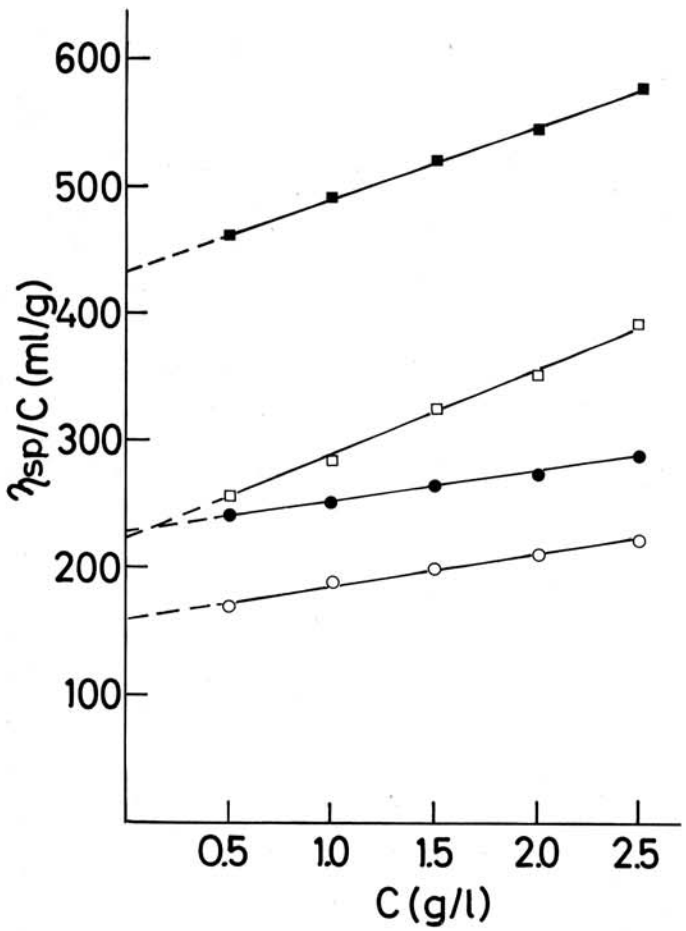

Fig. 1. Determination of the limiting viscosity numbers of kuzu and potato amylose and amylopectin. □ = kuzu amylose, o = kuzu amylopectin, ■ = potato amylose, • = potato amylopectin.

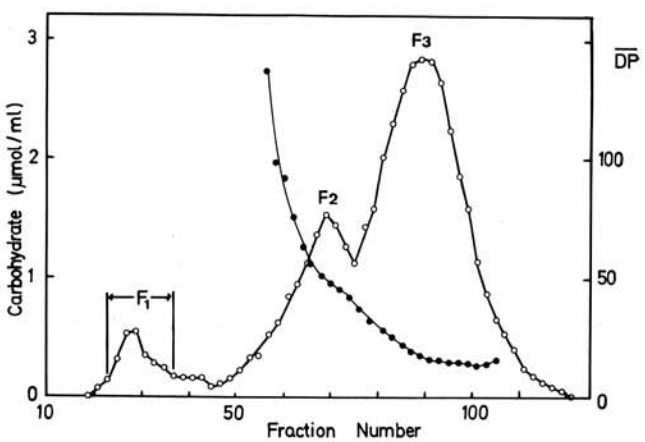

Fig. 2. Bio-Gel P-30 gel filtration pattern of debranched kuzu amylopectin, showing elution fractions F_1–F_3. • = Degree of polymerization ($\overline{DP}$), o = carbohydrate, as glucose.

Takeda and Hizukuri 1980), and they eluted much faster than the corresponding neutral saccharides of similar $\overline{dp}$ from a column packed with either Bio-Gel or Sephadex. Debranched waxy rice starch containing a trace amount of ester phosphate (Tabata et al 1975) gave only faintly observable F_1, whereas the debranched potato amylopectin gave a remarkable amount of it (Takeda and Hizukuri 1978).

Structure of Granules

Figure 3 shows a photomicrograph of kuzu starch. Spherical, hemispherical, and polygonal granules up to 30 μm in diameter can be seen, and the average granule size is approximately 15 μm in diameter. The X-ray diffraction pattern of kuzu starch was the C type, which resembled the type A rather than the B; very weak 15.8, 6.3, 4.0, and 3.7 Å diffraction lines were observed which is characteristic of the type B pattern (Fig. 4). Previously, Hizukuri and Nikuni (1957) reported that kuzu starch produced in Nara was a typical type C (a mixture of nearly equal amounts of types A and B). The crystalline structure of the starch used in this study seems to be shifted slightly toward type A, presumably because it was grown at the higher temperatures of southernmost Kyushu. The type C starch is highly susceptible to environmental temperature, as indicated in the starches of soy bean (Hizukuri et al 1961, 1965) and sweet potato (Nikuni et al 1963).

Pasting Properties

Kuzu starch showed a fairly stable paste viscosity during both heating and cooling in a Brabender amylograph (Fig. 5), confirming the results of Aoki and Tani (1975). The viscosity was

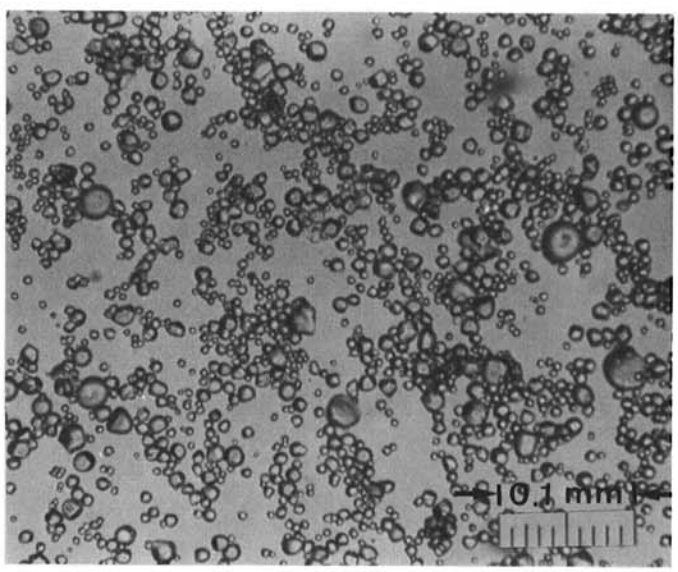

Fig. 3. Photomicrograph of kuzu starch.

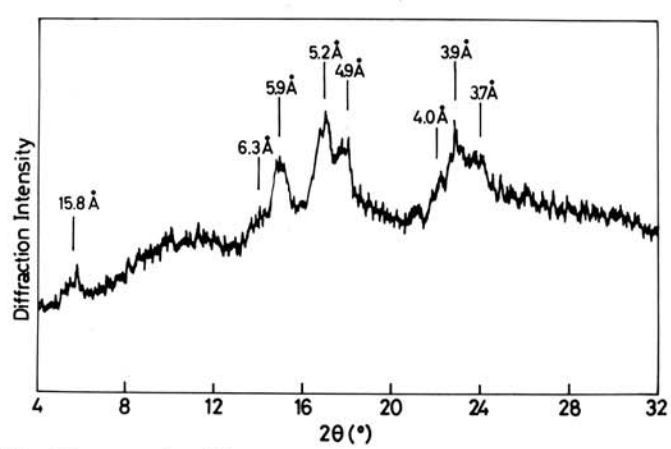

Fig. 4. X-ray powder diffractogram of kuzu starch.

low (maximum viscosity 245 BU at 6% concentration) compared to that of the starches of tubers and roots. The pasting temperature was 70° C under the test conditions. The profile of the amylogram differed from those of potato and tapioca starches. The amylogram of kuzu starch during the heating was similar to that of wheat starch, having a low and stable viscosity. 3-Methyl-1-butanol (3-MB) had little influenced on the viscosity of kuzu starch and lowered the pasting temperature to 65.5° C (Fig. 4). This response to 3-MB is similar to that of sweet potato starch but differs from those from wheat and potato starches. A pasting in 5% aqueous 3-MB causes a marked increase of viscosity of wheat and other cereal starches (Hizukuri and Takeda 1978), a considerable decrease in viscosity of potato and lily starches, and almost no change for sweet potato starch.[3]

Figure 6 shows the temperature-dependent pasting measured by the DG. The pasting started approximately at 45° C and progressed slowly up to 55° C and then rapidly up to 70° C. The pasting was notable only for DG-g at the onset (45° C), suggesting that the amylopectin played a leading role in the pasting. This has been observed in other starches–wheat, corn, potato, and sweet potato, but not rice (Suzuki and Hizukuri 1979, Takeda and Hizukuri 1974). At higher temperature (above 50° C), amylose and amylopectin appear to be pasted simultaneously, since DG-g and DG-i were nearly the same value. This pasting behavior is characteristic of kuzu starch and differs from that of potato, sweet potato, corn, and wheat starches, which show considerably larger DG-g than DG-i during heating (Takeda and Hizukuri 1974).

Retrogradation

The progress of retrogradation of the pastes of kuzu and potato starches on aging at 0° C was examined comparatively (Table III). The decrease of DG was rapid within the first hour and then became slow in both pastes. Kuzu starch paste showed a lower retrogradation tendency for DG-g than did potato starch paste; however the kuzu was higher for DG-i. This suggests that kuzu amylopectin is more resistant to retrogradation but the amylose is less resistant than that of potato starch. The shorter chain length of kuzu amylopectin than of potato may be the reason for the lower retrogradation tendency. The higher retrogradation tendency of kuzu amylose may be because of the smaller size (Lansky et al 1949). According to Whistler (1953), larger molecular weight amylose retrogrades more slowly, presumably because of steric effects resulting from the more coiled or convoluted structure. The relationship between branched structure and its retrogradation behavior should be examined in further studies because amylose appears to have a minor degree of branching (French 1975).

In a previous paper (Suzuki and Hizukuri 1979), we suggested that cooling aqueous pastes of potato starch for a short period caused some strong molecular associations of amylose that were not fully reversed upon heating at 100° C. This is worthy of study in connection with confectionery and noodle manufacture in which kuzu starch is preheated and cooled and then heated or steamed again for the final products. Table IV shows the effects of preheating and cooling on the reheating of kuzu starch pastes. The paste fully cooked on preheating, KP-100, showed remarkable retrogradation of amylose during a short period of cooling at 0° C, as estimated by decreases of DG-i, and stayed at a low DG-i (61%) on reheating. On the other hand, KP-100 maintained a high gelatinization state for DG-g (93–94%) on cooling and reheating. A similar, but lower, retrogradation of amylose was observed for KP-80, the paste partially cooked on preheating. These results suggest that the heat-resistant parts are formed by the retrogradation of amylose. Approximately 30% of the amylose remained in the uncooked state on preheating in KP-80, and this appears to be the main reason why it showed better pasting on reheating than did KP-100—in spite of possible participation of heat-moisture effect (Sair 1964) on the formation of the heat-resistant structure of KP-80, as suggested in potato starch (Suzuki and Hizukuri 1979). These results imply that the conditions for preheating and cooling have significant effect on the quality of the product by causing strong molecular association of amylose and are worthy of examination for improving both the quality and the control of processing in starchy foods manufacture.

[3]S. Hizukuri and C. Takeda, unpublished results.

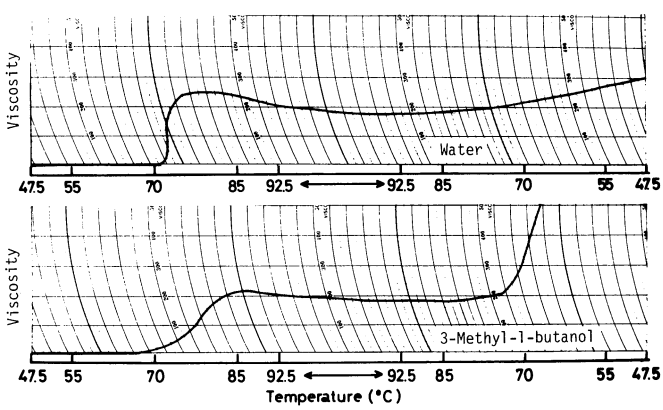

Fig. 5. Brabender amylograms of kuzu starch (6%). Pasting with water (**upper**) and with 5% 3-methyl-1-butanol (**lower**).

TABLE III
Retrogradation Tendencies (%) of Kuzu and Potato Starch Pastes[a] Stored at 0° C

Stored Time (hr)	Potato		Kuzu	
	DG-g[b]	DG-i[c]	DG-g	DG-i
0	100	98	100	98
1	87	68	93	49
15	84	68	92	46
24	83	68	91	46
72	83	48	91	45
120	79	46	89	11

[a] Pastes were prepared by heating 5% starch suspensions at 100° C for 20 min.
[b] Degree of gelatinization measured with glucoamylase.
[c] Degree of gelatinization measured with iodine titration.

TABLE IV
Effect of Preheating and Cooling[a] on Final Reheating of Kuzu Starch Paste

Paste[b]	Preheating		Cooling		Reheating	
	DG-g[c]	DG-i[d]	DG-g	DG-i	DG-g	DG-i
KP-100	100	98	93	49	94	61
KP-80	80	73	70	65	96	81

[a] Cooling by standing the preheated pastes at 0° C for 1 hr; reheating by heating the cooled pastes at 100° C for 20 min.
[b] Pastes KP-100 and KP-80 were prepared by heating 5% aqueous kuzu starch suspensions (100 ml) at 100 and 66.5° C, respectively, for 20 min.
[c] Degree of gelatinization (%) measured by glucoamylase.
[d] Degree of gelatinization (%) measured by iodine titration.

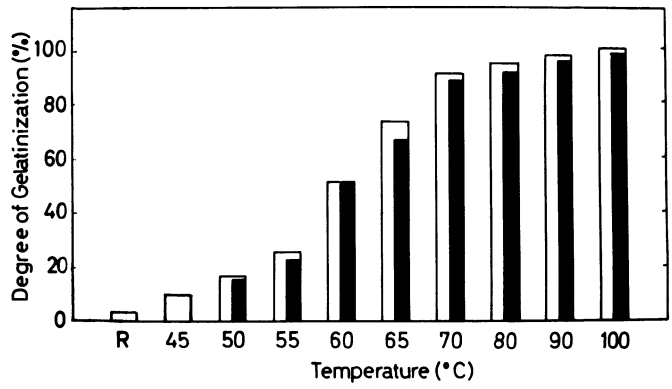

Fig. 6. Temperature-dependent gelatinization measured by the degree of gelatinization. □ = glucoamylase digestion method, ■ = iodine titration method, R = raw starch.

ACKNOWLEDGMENTS

We thank T. Harada for the gift of *Pseudomonas* isoamylase. This work was supported in part by Grant 55603 to S. Hizukuri from the Ministry of Education and Culture of Japan.

LITERATURE CITED

AOKI, M., and TANI, Y. 1975. Chemical and physical properties of bracken and arrowroot starches. J. Home Econ. (Tokyo) 26:249.

ALLEN, R. J. L. 1940. The estimation of phosphorus. Biochem. J. 34:858.

BAILEY, J. L. 1967. Techniques in Protein Chemistry. Elsevier Pub.: Amsterdam.

BANKS, W., and GREENWOOD, C. T. 1966. The fine structure of amylose: The action of pullulanase as evidence of branching. Arch. Biochem. Biophys. 117:674.

BANKS, W., and GREENWOOD, C. T. 1967. Physicochemical studies on starches. XXXII. The incomplete β-amylolysis of amylose: A discussion of its cause and implications. Staerke 19:197.

BANKS, W., and GREENWOOD, C. T. 1975. Starch and Its Components. Edinburgh Univ. Press: Edinburgh.

BAUM, H., and GILBERT, G. A. 1954. The anaerobic dispersion of potato starch. Oxygen-sensitive bonds. Chem. Ind. 489.

COWIE, J. M. G., and GREENWOOD, C. T. 1957a. Physicochemical studies on starches. VI. Aqueous leaching and the fractionation of potato starch. J. Chem. Soc. 2862.

COWIE, J. M. G., and GREENWOOD, C. T. 1957b. Physicochemical studies on starches. VIII. Further observations on the fractionation of potato starch. J. Chem. Soc. 4640.

FISKE, C. H., and SUBBAROW, Y. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375.

FRENCH, D. 1975. Chemistry and biochemistry of starch. Page 267 in: Whalan, W. J., ed. Biochemistry of Carbohydrates. Butterworths University Park Press: London.

GREENWOOD, C. T. 1970. Starch and glycogen, Vol. IIB. Page 471 in: Pigman, W., and Horton, D., eds. The Carbohydrates, Chemistry and Biochemistry. Academic Press: New York and London.

GUNJA-SMITH, Z., MARSHALL, J. J., MERCIER, C., SMITH, E. E., and WHELAN, W. J. 1972. A revision of the Meyer-Bernfeld model of glycogen and amylopectin. FEBS Lett. 12:101.

HIZUKURI, S., FUJII, M., and NIKUNI, Z. 1961. Effect of temperature during germination on the crystalline type of starch in soybean seedlings. Nature (London) 192:239.

HIZUKURI, S., FUJII, M., and NIKUNI, Z. 1965. Studies on starch of soybean seedlings. Staerke 17:40.

HIZUKURI, S., and NIKUNI, Z. 1957. X-ray diffractometric studies on starches. II. Structure of "C"-type crystallite. Nippon Nogei Kagaku Kaishi 31:525.

HIZUKURI, S., and OSAKI, S. 1978. A rapid Smith-degradation for the determination of non-reducing, terminal residues of (1➔4)-α-D-glucans. Carbohydr. Res. 63:261.

HIZUKURI, S., TABATA, S., and NIKUNI, Z. 1970. Studies on starch phosphates. I. Estimation of glucose 6-phosphate residues in starch and the presence of other bound phosphates. Staerke 22:338.

HIZUKURI, S., and TAKEDA, C. 1978. Studies on the gelatinization of starches. II. Effect of lower alcohols on the gelatinization of cereal starches. Staerke 30:228.

HIZUKURI, S., TAKEDA, Y., USAMI, S., and TAKASE, Y. 1980. Effect of aliphatic hydrocarbon groups on the crystallization of amylodextrin. Carbohydr. Res. 83:193.

KATO, K., KONISHI, Y., AMEMURA, A., and HARADA, T. 1977. Affinity chromatography of *Pseudomonas* isoamylase on cross-linked amylose gel. Agric. Biol. Chem. 41:2077.

KJØLBERG, O., and MANNERS, D. J. 1963. Studies on carbohydrate-metabolizing enzymes. IX. The action of isoamylase on amylose. Biochem. J. 86:258.

KOEHLER, L. H. 1952. Differentiation of carbohydrates by anthrone reaction rate and color intensity. Anal. Chem. 24:1576.

LANSKY, S., KOOI, M., and SCHOCH, T. J. 1949. Properties of the fractions and linear subfractions from various starches. J. Am. Chem. Soc. 71:4066.

LARSON, B. L., GILLES, K. A., and JENNES, R. 1953. Amperometric method for determining the sorption of iodine by starch. Anal. Chem. 25:802.

LII, C-Y., and LINEBACK, D. R. 1977. Characterization and comparison of cereal starches. Cereal Chem. 54:138.

NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375.

NIKUNI, Z., HIZUKURI, S., FUJII, M., DOI, K., HASEGAWA, H., MORIWAKI, T., NARA, S., and MAEDA, I. 1963. Effect of cultivating soil temperature on the properties of sweet potato starch. Nippon Nogei Kagaku Kaishi 37:673.

SAIR, L. 1964. Heat-moisture treatment of starches, Vol. IV. Page 283 in: Whistler, R. L., ed. Methods in Carbohydrate Chemistry. Academic Press: New York.

SCHOCH, T. J. 1942. Non-carbohydrate substances in the cereal starches. J. Am. Chem. Soc. 64:2954.

SOMOGYI, M. 1952. Notes on sugar determination. J. Biol. Chem. 195:19.

SUZUKI, A., and HIZUKURI, S. 1979. Basic studies on cooking potatoes. II. Effect of potato extract on the interrelation of gelatinization-retrogradation of potato starch. Cereal Chem. 56:257.

SUZUKI, H., NAGABORI, T., and SAITO, T. 1958. Comparison of some properties of sweet potato starch and kuzu starch. Denpun Kogyo Gakkaishi 6:24.

TABATA, S., and HIZUKURI, S. 1971. Studies on starch phosphate. II. Isolation of glucose 3-phosphate and maltose phosphate by acid hydrolysis of potato starch. Staerke 23:267.

TABATA, S., NAGATA, K., and HIZUKURI, S. 1975. Studies on starch phosphate. III. On the esterified phosphates of some cereal starches. Staerke 27:333.

TAKEDA, C., and HIZUKURI, S. 1974. Characterization of the heat dependent pasting behavior of starches. Nippon Nogei Kagaku Kaishi 48:663.

TAKEDA, Y., and HIZUKURI, S. 1969. Improved method for crystallization of sweet potato β-amylase. Biochem. Biophys. Acta 185:469.

TAKEDA, Y., and HIZUKURI, S. 1978. Location of phosphate in potato amylopectin. Page 145 in: Abstracts of Agric. Chem. Soc. Meeting, Nagoya, Japan. Agric. Chem. Soc.: (Tokyo)

TAKEDA, Y., and HIZUKURI, S. 1981. Re-examination of the action of sweet potato β-amylase on phosphorylated (1➔4)-α-D-glucan. Carbohydr. Res. 89:174.

WHISTLER, R. 1953. Starch retrogradation, Page 213 in: Radley, J. A., ed. Starch and Its Derivatives, Vol. 1, 3rd ed. Chapman and Hall: London.

[Received August 18, 1980. Accepted November 24, 1980]