Physicochemical Properties of Starches Extracted from Sweet Potato Roots Differing in Physiological Age

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The physicochemical properties of starches from sweet potato roots differing in stages of development and tissue zone were examined. All of the sweet potato starch granules had both spherical and polygonal shapes by scanning electron microscopy observation. All starches gave a Ca-type X-ray diffraction pattern. Pasting properties were also characterized by a rapid viscoanalyzer. Pasting temperatures were somewhat higher in the peel starch and were lower in the late stage of development. Peak viscosity and breakdown tended to be higher with increasing physiological age. The ratio of short chains to long chains of amylopectin determined by gel permeation chromatography (GPC) of the debranched starch was slightly higher in the early stage of development but was similar among different tissue zones. The distributions of chain length (degree of polymerization 6–17) of these amylopectins calculated from GPC substantially corresponded to those from high-performance anion exchange chromatography.

Keywords: *Sweet potato; starch; physiological age; SEM observation; X-ray diffraction; RVA analysis; amylopectin chain length*

INTRODUCTION

Starch properties are altered according to the botanical origin of the starch. Furthermore, starches from the same botanical origin have different characteristics depending on the cellular conditions of the plant tissue from which the starch is prepared. In recent studies (Noda et al., 1992a,b, 1995), to obtain knowledge of starch granules from the same plant species differing in physiological age, we have researched some basic characteristics, namely granule size distribution, amylose content by the blue value method, gelatinization properties by differential scanning calorimetry (DSC), enzymatic digestibility by glucoamylases, and distributions of the short chains of amylopectins [degree of polymerization (DP) 6–17] by high-performance anion exchange chromatography (HPAEC) of starches extracted from sweet potato roots differing in stages of development and tissue zone. From these studies, we found that granule size distribution and enzymatic digestibility changed distinctively according to the difference in physiological age. Starch granules extracted from immature roots and the cambium, whose cells are physiologically younger, had a smaller size and higher digestibility by glucoamylases (Noda et al., 1992a,b). On the contrary, the amylose content (Noda et al., 1992a,b) and distributions of amylopectin chain length (Noda et al., 1995) remained almost constant with physiological age. There have been some studies other than ours on the properties of starches derived from sweet potato roots differing in physiological age (Fujimoto et al., 1971a,b; Kainuma et al., 1984). However, few studies have completely evaluated the properties of these starches.

Our present study was undertaken to obtain more information on the effect of physiological conditions of the sweet potato roots on the properties of starch granules. For this purpose, the physicochemical properties unexamined in our previous papers on two varieties of sweet potato roots differing in stages of development and tissue zone were determined. We examined scanning electron microscopy (SEM) images, X-ray diffraction patterns and pasting properties by rapid viscoanalyzer (RVA) of these starches. Gel permeation chromatography (GPC) of the debranched components of these starches with isoamylase was also performed to investigate the fine structure of the amylopectins of the starches. In addition, we compared the chain length distributions (DP 6–17) of these amylopectins determined by GPC with those by HPAEC.

MATERIALS AND METHODS

Materials. Sweet potato roots [Koganesengan (KS) and Shiroyutaka (SY) varieties], which were harvested during three stages of development, namely on July 24 (62 days after planting), September 2 (102 days after planting), and October 7 (137 days after planting), 1991 (Noda et al., 1992b), were used for developmental difference tests. The KS and SY sweet potato roots harvested on October 7 (137 days after planting), 1991, and separated into the peel (P), cambium (C) and inner tissue (IT) (Noda et al., 1992a) were used for zonal difference test. Sweet potato starch was isolated from each sample as reported previously (Noda et al., 1992c). Isoamylase of *Pseudomonas amyloderamosa* was obtained from Seikagaku Kogyo Co., Tokyo, Japan.

SEM Observation. Starch samples (KS only) were sprinkled onto double-faced tape attached to specimen stubs and coated with Pt. The mounted samples were viewed with a Hitachi S-4000 scanning electron microscope (Hitachi Co., Tokyo, Japan) at an accelerating voltage of 15 kV. Electromicrographs of each starch were taken at a magnification of 1000×.

X-ray Diffraction. The X-ray diffraction test was conducted on a Mini Flex X-ray diffractometer (Rigaku-denki Co., Tokyo, Japan) under the following conditions: X-ray tube, 30 kV, 10 mA; scanning speed, 2/min; divergence slit, 1; receiving slit, 0.3 mm; range, 1000 cps; chart speed, 4 mm/min; time constant, 2 s; irradiation, Cu Kα; eliminating, Kβ with a Ni filter.

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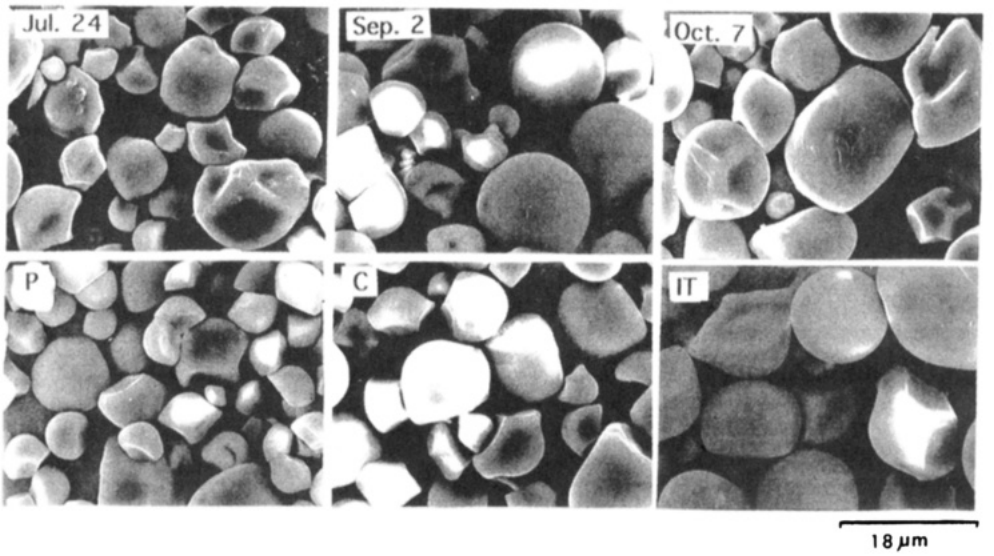

Figure 1. SEM images of starches from KS differing in physiological age (×1000). (This figure is reproduced at 60% of its original size.)

Pasting Properties by RVA. The pasting properties of starch suspensions (10% w/v, dry weight basis, 25 mL) were studied using the RVA-3D (Newport Scientific Pty. Ltd., Australia). The temperature was raised from 30 to 95 °C at a rate of 5 °C/min, kept at 95 °C for 6min, and lowered to 50 °C at the same rate and held for 10 min.

GPC of Debranched Starch. The treatment of starches with *Pseudomonas* isoamylase was carried out as described (Noda et al., 1995). Aliquots (10 mL) of debranched components containing approximately 10 mg of polysaccharide were loaded on a Toyopearl HW-50SF column (2.6 cm × 100 cm) and eluted with distilled water. Fractions (5 mL) were collected at 7.5 min intervals. The total sugar content of each fraction was measured according to the phenol–sulfuric acid method (Dubois et al., 1956). The eluted components were separated into three fractions, namely fractions 1, 2, and 3, which were divided at minimum points between the fractions according to total sugar content. The weight percentages of fractions 1–3 were calculated from each fraction area. Reducing capacity was determined according to the Park–Johnson method as modified by Hizukuri et al. (1981). The DP were calculated from the amounts of total sugar and reducing capacity. For the fractions of DP 6–17, the ratio of the relative weight content of each fraction to the sum of all fractions of DP 6–17 was calculated to obtain the chain length (DP 6–17) distributions of amylopectins (KS only). The distributions of amylopectins by GPC calculated in this way were compared with those determined by HPAEC in our previous paper (Noda et al., 1995).

RESULTS AND DISCUSSION

As shown in Figure 1, the morphology of the starch granules extracted from sweet potato (KS) roots of differing stages of development and tissue zone was determined using SEM. All starch granules had both spherical and polygonal shapes, irrespective of the physiological age of the tissue of the roots from which the starch was isolated. According to Fujimoto et al. (1971a), who studied the morphology of sweet potato starch granules of the P, C, and IT by light microscopy, the shapes of these starch granules were similar. On the basis of these results, the morphology of the starch granules is not influenced by the cellular conditions of sweet potato roots. Starches from the immature roots (i.e., harvested on July 24), P, and C had significantly smaller granule size. In our previous studies, granule size distributions of these starches were determined

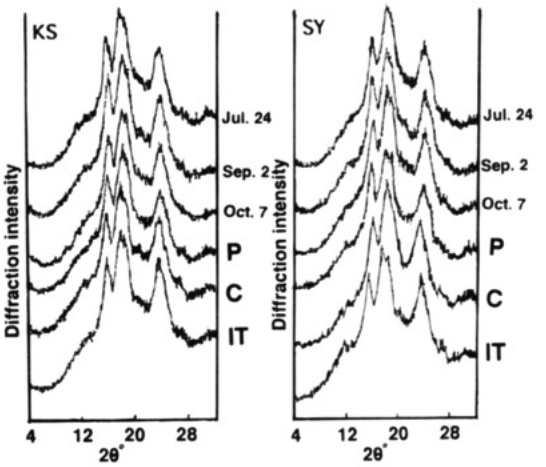

Figure 2. X-ray diffraction patterns of starches from KS and SY differing in physiological age.

using an image analyzer attached to a light microscope. We showed a significant increase in average granule sizes from 8.58 to 11.0 μm in the variety KS during root formation (Noda et al., 1992b). There was a difference in average size distribution (P, 9.51 μm; C, 8.58 μm; IT, 12.5 μm) of starches from different tissue zones of KS (Noda et al., 1992a). These results were in good agreement with our present ones. Our data to date demonstrate that the size of the starch granules becomes larger with increasing physiological age.

X-ray diffraction patterns of starch granules derived from sweet potato roots differing in stages of development and tissue zone are presented in Figure 2. Starch granules at three different stages of development exhibited the Ca-type (C-type near A-type). The X-ray diffraction patterns of starch granules are classified into three types, A-, B-, and C-types. The C-type has been suggested to be a mixture of A- and B-types. These crystalline varieties of starch granules appear to be specific for their botanical origin. In general, the A-type has been found in cereal starches and B-type in tuber starches. Previous studies indicated that the X-ray diffraction patterns were between A-type and Cb-type (C-type near B-type) for a number of sweet potato varieties (Hizukuri et al., 1983; Shiotani et al., 1991; Takeda et al., 1986), which agrees with our findings.

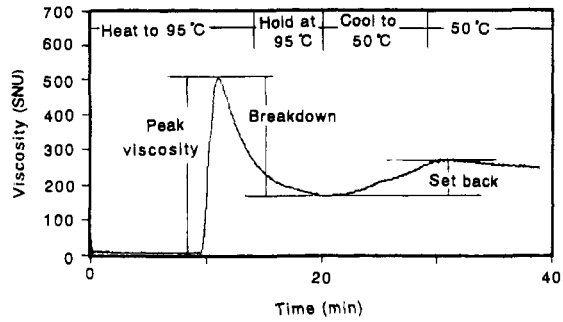

Figure 3. RVA viscogram of starch from KS harvested on October 7 (10% w/v, dry weight basis).

Table 1. Pasting Properties by RVA of Starches of KS and SY Differing in Physiological Age[a]

variety	day of harvest or tissue zone[b]	peak viscosity (SNU)	breakdown (SNU)	setback (SNU)	pasting temp (°C)
KS	July 24	469	245	117	73.4
	Sept 2	454	293	95	73.5
	Oct 7	504	336	99	72.9
	P	492	261	124	74.5
	C	477	302	107	72.0
	IT	516	366	108	72.9
SY	July 24	438	213	143	75.4
	Sept 2	491	332	105	74.3
	Oct 7	533	371	108	73.8
	P	538	330	106	74.9
	C	544	357	108	74.2
	IT	597	440	109	73.5

[a] Values are the average of two determinations. [b] P, peel; C, cambium; IT, inner tissue.

X-ray diffraction patterns alter depending on the environmental temperature for C-type starches, such as sweet potato (Nikuni et al., 1963) and soybean (Hizukuri et al., 1961). It was found that the X-ray diffraction patterns of these starches shifted from A-type to B-type with decreasing environmental temperature. On the contrary, temperature-dependent changes in the X-ray diffraction patterns have not been observed in the A- and B-type starches (Hizukuri, 1969). In our experiment, temperature-dependent transition in the X-ray diffraction patterns was not found. For this reason, the environmental temperature on October 7 was assumed to be too high to influence the X-ray diffraction pattern significantly. In the zonal test, the X-ray patterns were also Ca-type in all cases. According to Fujimoto et al. (1971a), the C and IT starches of sweet potato tended to shift the structure toward B- and A-type, respectively, unlike our data.

Amylograph has been utilized for characterizing the pasting properties of starch. However, it needs a relatively large amount of sample (15–50 g) for analysis. The advantage of RVA is the small sample requirement (1–3 g) for analysis. Having only a limited amount of starch sample, RVA was used in this study for the determination of starch pasting properties. Figure 3 shows the RVA curve of the starch of KS harvested on October 7 at 10% concentration (w/v, dry weight basis). Peak viscosity, breakdown, and setback are defined in this figure. The viscosity was defined as (stirring number unit) (SNU). One SNU is equivalent to 0.1 cP. The pasting properties by RVA of starches of sweet potato roots differing in stages of development and tissue zone are shown in Table 1. Suzuki et al. (1993) studied the pasting properties of starches from some plant species, including two sweet potato varieties, KS

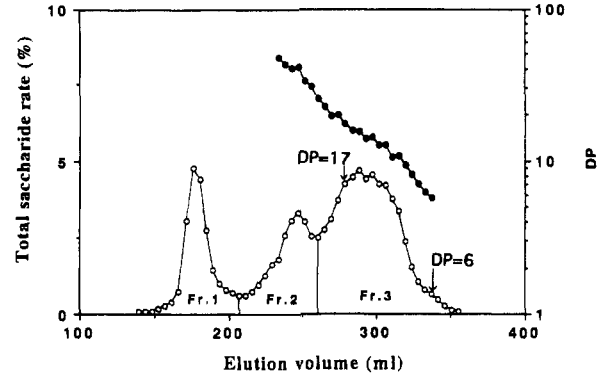

Figure 4. Toyopearl HW-50SF gel permeation chromatogram of debranched starch from KS harvested on October 7. Total sugar (○) and DP (●) were measured as described under Materials and Methods.

and Minamiyutaka (MY), by RVA at 6% concentration (w/v dry weight basis). They observed that the pasting temperatures were 74.0 and 75.5 °C for KS and MY, respectively, which is in conformity with our data. Peak viscosities were shown to be 106 SNU (KS) and 122 SNU (MY), which are lower than that of potato starch (409 SNU) and similar to that of arrowhead (113 SNU) and tapioca (107 SNU) starches. These values are much lower than ours because the research was carried out at lower starch concentration. Pasting temperatures of the P starches were somewhat higher (KS, 74.5 °C; SY, 74.9 °C) than those of the C (KS, 72.0 °C; SY, 74.2 °C) and IT (KS, 72.9 °C; SY, 73.5 °C) starches. We observed that the onset temperatures (T_0) of P starches determined by DSC were higher (KS, 72.8 °C; SY, 73.9 °C) than those of C (KS, 69.3 °C; SY, 72.5 °C) and IT (KS, 72.1 °C; SY, 72.4 °C) starches (Noda et al., 1992a), which is consistent with the results of pasting temperatures determined by RVA. Pasting temperatures were slightly low in the later stage of development of sweet potato roots. The same trend was observed in our previous results for T_0 determined by DSC (Noda et al., 1992b). A decrease in soil temperature during the later stage of development of sweet potato roots would result in changes in temperatures for gelatinization by both RVA and DSC. Peak viscosities varied from 454 to 504 SNU (KS) and from 438 to 533 SNU (SY) at all stages of development, displaying the highest value on October 7. Breakdown was also found to increase during sweet potato root formation and reached 336 and 371 SNU on October 7 for KS and SY, respectively. Similar increases in peak viscosity and breakdown during development of sweet potato roots were observed using the amylograph (Fujimoto et al., 1971b). The IT starches gave higher peak viscosity (KS, 516 SNU; SY, 597 SNU) and breakdown (KS, 366 SNU; SY, 440 SNU) than P and C starches. The results were in good agreement with those of Fujimoto et al. (1971a), who studied zonal differences in pasting properties of sweet potato starches by amylograph. Our experiment demonstrated that peak viscosity and breakdown become higher with increasing physiological age. Setback, which is the recovery of viscosity by cooling after the starch paste has been heated, was highest in the earliest stage of development. The P starch of KS displayed a slightly higher breakdown (124 SNU). However, such a phenomenon was not observed in the case of SY.

Figure 4 shows the GPC elution profile of the isoamylase-debranched starch from KS harvested on October 7. Fraction 1 is considered to be mostly amylose. Fractions 2 and 3 correspond to the linear long and short

Table 2. Chain Length Distributions by GPC of Debranched Starches of KS and SY Differing in Physiological Age

variety	day of harvest or tissue zone[a]	fraction 1	2	3	3/2	amylose content[b] (%)
KS	July 24	20.2	21.7	58.1	2.68	19.7
	Sept 2	18.4	25.5	56.1	2.20	20.4
	Oct 7	19.9	24.6	55.5	2.26	20.4
	P	18.8	25.8	56.0	2.17	19.8
	C	18.9	24.1	57.0	2.37	20.4
	IT	18.5	24.1	57.4	2.38	20.2
SY	July 24	22.8	22.1	55.1	2.49	21.9
	Sept 2	20.7	25.7	53.7	2.09	23.1
	Oct 7	22.3	25.9	51.8	2.00	22.8
	P	20.0	24.4	55.6	2.27	22.9
	C	21.1	26.4	52.4	1.98	23.7
	IT	20.2	26.3	53.5	2.03	22.6

[a] See footnote *b* of Table 1. [b] Data are from our previous papers (Noda et al., 1992a,b). They were determined by the blue value at 680 nm. Values are the average of three determinations.

Table 3. ANOVA of the Ratio of Fraction 3 to Fraction 2

type of test	factor	DF	variance	*F* value
developmental test	variety	1	0.0580	18.7
	day of harvest	2	0.1248	40.3[a]
	error	2	0.0031	
zonal test	variety	1	0.0683	1.84
	tissue zone	2	0.0011	0.03
	error	2	0.0370	

[a] Significant at 5%.

unit-chains of amylopectin, respectively. Table 2 summarizes the distribution of isoamylase-debranched starches of sweet potato roots differing in stages of development and tissue zone. Complete recoveries of total sugar were recognized in all cases. Fraction 1 varied from 18.4 to 20.2% (KS) and from 20.0 to 22.8% (SY). Developmental and zonal differences in fraction 1 content were not observed to be noteworthy. The amylose contents calculated from the blue value at 680 nm in our previous papers (Noda et al., 1992a,b) are also presented in Table 2. It is clear that the amylose contents obtained from GPC data for debranched starches were similar to those from the blue value method. All sweet potato amylopectins used in this study were found to have a biomodel distribution of unit chains, as indicated previously (Takeda et al., 1986). The ratio of fraction 3 to fraction 2, which is regarded as an index of the degree of branching of amylopectin, was slightly higher on July 24, the earliest stage of development (KS, 2.68; SY, 2.49), which agreed with the report of Kainuma et al. (1986).

Table 3 summarizes the results of ANOVA for the ratio of fraction 3 to fraction 2. The factor "day of harvest" was found to influence the ratio of fraction 3 to fraction 2. These findings indicate that amylopectin from immature sweet potato roots had a slightly higher degree of debranching. In rice (Asaoka et al., 1985) and corn (Inouchi et al., 1984), it was shown that the developmental changes in the distribution of the unit chain length of amylopectin determined by GPC of isoamylase-debranched starches did not occur significantly. There was no substantial difference in the ratio of fraction 3 to fraction 2 among the tissues (Tables 2 and 3), indicating that zonal differences in the degree of branching of amylopectin did not exist in sweet potato. In some varieties of rice, elution profiles of debranched materials of the outer layer starches showed

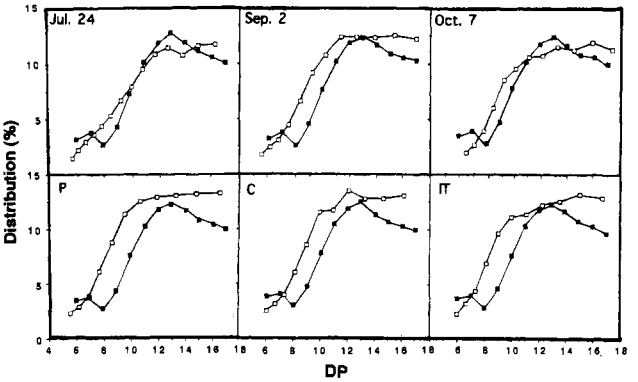

Figure 5. Chain length (DP 6–17) distributions of amylopectins from KS differing in stages of development (upper) and tissue zone (lower). GPC data (□) were determined as described under Materials and Methods. HPAEC data (■) are from our previous paper (Noda et al., 1995).

chain length distributions similar to those of the central core starches (He and Suzuki, 1989), as shown in our findings.

In the previous paper (Noda et al., 1995), we determined the maltosaccharides produced after debranching starches used in this study by HPAEC equipped with a pulsed amperometric detector. Maltosaccharides of DP 50 or higher were separated into individual fragments and a quantitative determination of these saccharides could be made up to DP 17. Using this method, the chain length (DP 6–17) distributions of these amylopectins were characterized, indicating that developmental and zonal changes in the distributions of amylopectins were not recognized. Thus, there have been various methods of examining the chain profile of amylopectin. However, no examinations were done concerning the comparison of these methods. For this reason, we tried to compare the chain length distributions of amylopectins determined by GPC with those by HPAEC. Results are shown in Figure 5. Our present data on the distribution of amylopectin chain length by HPAEC are expressed on a weight basis, not on a molar basis as reported in the previous paper (Noda et al., 1995). Although all amylopectins displayed a peak at DP 13 and had a valley at DP 8 by the HPAEC method, such trends were not observed in the case of GPC. The reason these differences were found was that the Toyopearl HW-SF50 gel was not able to resolve maltosaccharides thoroughly. However, the profiles obtained by these two methods were in substantial agreement.

In the present study, we described the effect of the physiological conditions of the sweet potato roots on the properties of starch granules. Additionally, the distributions of amylopectin chain length (DP 6–17) calculated from GPC were compared with those from HPAEC. The size of the granules, the peak viscosity, and breakdown changed due to the differences in physiological age, although the morphology of the granules and the X-ray diffraction pattern did not. The proportion of short chains to long chains of amylopectin determined by GPC of the isoamylase-debranched starch was slightly higher in the early stage of development, but the proportions were similar among different tissue zones. There was substantial similarity in the distributions of amylopectin chain length determined by GPC and HPAEC.

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