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Effect of soil temperature on starch properties of sweet potatoes

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Abstract

Starch was extracted from the tubers of two sweet potato cultivars (Ayamurasaki and Sunnyred) grown at four soil temperatures (15, 21, 27 and 33°C). The physicochemical properties of the isolated starches were analyzed in an attempt to investigate the impact of soil temperature on sweet potato starch. When the soil temperature increased from 15 to 33°C, the amylose content of the starch increased ca. 5.0%. The average granule size increased ca. 4.0 μm as the soil temperature increased from 15 to 27°C. High-performance anion-exchange chromatographic data indicated a distinct reduction in short chains of amylopectin with DP 6 and 7 with increasing soil temperatures. There were extensive variations in starch gelatinization characteristics as determined by differential scanning calorimetry. Large increases were found, values of onset (Ayamurasaki, 25.0°C; Sunnyred, 21.8°C) and peak (Ayamurasaki, 22.8°C; Sunnyred, 22.3°C) temperatures, as well as heat of gelatinization (Ayamurasaki, 5.4 J/g; Sunnyred, 3.3 J/g), as the soil temperature increased from 15 to 33°C. Starch pasting properties measured using the Rapid Viscoanalyzer varied according to soil temperature. Higher soil temperatures were generally associated with lower values of peak viscosity and setback. Thus, it was demonstrated that the soil temperature during the development of sweet potato tubers had an important influence on the starch properties.

Keywords: Soil temperature; Starch properties; Sweet potatoes

1. Introduction

Starch properties are affected by environmental factors, especially by growth temperature, in many plant species. In rice starch, lower air temperatures were found to increase the amylose content and the short-chain to long-chain ratios of amylopectin, in addition to lowering gelatinization temperatures (Asaoka, Okuno, Sugimoto & Fuwa, 1985). Tester, South, Morrison and Ellis (1991) indicated that an increased ambient temperature, from 10 to 20°C, resulted in a higher gelatinization temperature in barley starch. However, they found few differences in amylose content and in the fine structure of amylopectin with a change in ambient temperature. In wheat, amylose content tended to increase and gelatinization temperature was clearly higher as the environmental temperature increased during grain filling (Shi, Seib & Bernardin, 1994; Tester et al., 1995). Moreover, Lu, Jane, Keeling and Singletary (1996) studied the effects of the temperature when the ear developed on maize starch properties. They observed a smaller starch granule size, higher amylose content and higher gelatinization temperature when the temperature was raised from 25 to 35°C.

In our previous investigation (Noda, Takahata, Sato, Ikoma & Mochida, 1997), we found clear differences in the physicochemical properties of starches in sweet potato tubers with different planting and harvesting dates. The results showed that late planting and late harvesting led to a higher gelatinization temperature and higher peak viscosity. The degree of branching of amylopectin was highest when harvesting was early. Generally, in Japan, cuttings of sweet potatoes are planted in late spring, and tubers are harvested in mid-autumn, when the temperature is gradually falling. Therefore, late planting and late harvesting represent lower soil temperatures during sweet potato development. The results of our previous investigation suggested that the soil temperature during sweet potato development would affect starch properties. According to the early report of Hizukuri (1969), pasting temperature apparently increased, peak viscosity, amylase digestibility and phosphate content decreased, and the X-ray diffraction pattern shifted to the B-type with increasing soil temperature during sweet potato development. However, for sweet potatoes, the effects of environmental temperature on starch properties have yet been fully elucidated.

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Table 1
Starch content and amylose content in sweet potatoes grown at four different soil temperatures

Cultivar	Soil temperature (°C)	Starch content (%)[a]	Amylose content (%)[b]
Ayamurasaki	15	20.4	12.8
	21	26.9	14.6
	27	31.8	15.8
	33	21.9	17.3
Sunnyred	15	20.9	15.6
	21	31.5	16.8
	27	27.4	19.9
	33	26.8	20.6

[a] Values are means of four determinations. Standard deviation ±1.4%.
[b] Values are means of three determinations. Standard deviation ±0.6%.

This investigation was conducted to elucidate the precise relationships between soil temperature during sweet potato development and the starch properties. For this purpose, we used two sweet potato cultivars grown under controlled environmental conditions and analyzed their physicochemical properties.

2. Materials and methods

2.1. Starch samples

The purple-fleshed cultivar, Ayamurasaki, and the orange-fleshed cultivar, Sunnyred, were used in this study. The cultivars were grown in a temperature-controlled greenhouse at the Kyushu National Agricultural Experiment Station at Miyakonojo, Miyazaki. The cuttings were planted on June 6, 1997, and grew for 31 days in a soil temperature of 25°C and air temperatures of 28°C day/23°C night. Then the samples were further maintained for 99 days at four levels of soil temperature (15, 21, 27 or 33°C) while the air temperature remained unchanged. Starch granules were isolated from each sample as reported earlier (Noda et al., 1997).

2.2. Analytical methods

The starch content was determined using the modified method of McCready, Guggolz, Silviera and Owens (1950), as described previously (Noda, Takahata & Nagata, 1992b). Starch granule size was measured by an image analyzer (Excel-II, Nippon Avionics Co., Tokyo, Japan) attached to a light microscope (Microphot-FXA, Nikon Co., Tokyo, Japan) on approximately 1200 granules, as described elsewhere (Noda, Takahata, Nagata & Monma, 1992a). Amylose content was determined from the blue value at 680 nm according to the method reported previously (Noda et al., 1992a). Enzymatic digestibility of the raw starch by crystalline glucoamylase of *Rhizopus niveus* was conducted for 4 h at 40°C with a substrate concentration of 2% (Noda et al., 1992b). Each starch was debranched by *Pseudomonas amyloderamosa* isoamylase, as described earlier (Noda, Takahata & Sato, 1995). The linear maltosaccharides produced after debranching were analyzed by high-performance anion-exchange chromatography (HPAEC) using a Dionex BioLC system (Dionex Co., Sunnyvale, CA) equipped with pulsed amperometric detection (PAD) and a CarboPac PA1 Column (4 × 250 mm), as Koizumi, Fukuda and Hizukuri (1991) reported. The area of each peak of linear chains up to DP 45 was determined using a Hitachi D-2500 Chromato-integrator. Differential scanning calorimetry (DSC) measurements were carried out using a Perkin–Elmer DSC-7 analyzer (Perkin–Elmer Co., Norwalk, CT) equipped with a 1020 TA workstation (Noda, Takahata, Sato, Ikoma & Mochida, 1996; Noda, Takahata, Sato, Kumagai & Yamakawa, 1998a). Water was added to approximately 3 mg of starch (dry-weight basis) in an aluminum pan to give a starch concentration of 30% (dry-weight basis). The pan was hermetically sealed and heated from 30 to 100°C at a heating rate of 10°C/min. T_o, T_p and ΔH were recorded. Rapid visco-analyzer (RVA) measurements were conducted using the RVA-3D (Newport Scientific Pvt. Ltd., Australia) as follows. The starch suspension (8% w/v, dry-weight basis, 25 ml) was kept at 50°C for 1 min, heated to 95°C at 13.2°C/min, kept at 95°C for 2.7 min, and then cooled to 50°C at 11.6°C/min, and kept at 50°C for 2 min. The viscosity was measured in RVA units (RVU).

3. Results

Starch content was in the range of 20.4–31.8% and 20.9–31.5% for Ayamurasaki and Sunnyred, respectively (Table 1). It was lowest in the samples grown at 15°C and relatively low in those grown at 33°C. Amylose content from the blue-value method varied from 12.8 to 17.3% and from 15.6 to 20.6% for Ayamurasaki and Sunnyred, respectively (Table 1). Distinct increases in amylose content (Ayamurasaki, 4.5%; Sunnyred, 5.0%) were observed when the soil temperature was raised from 15 to 33°C.

The distributions of starch granule size, measured by an imaging analyzer attached to a light microscope, are shown

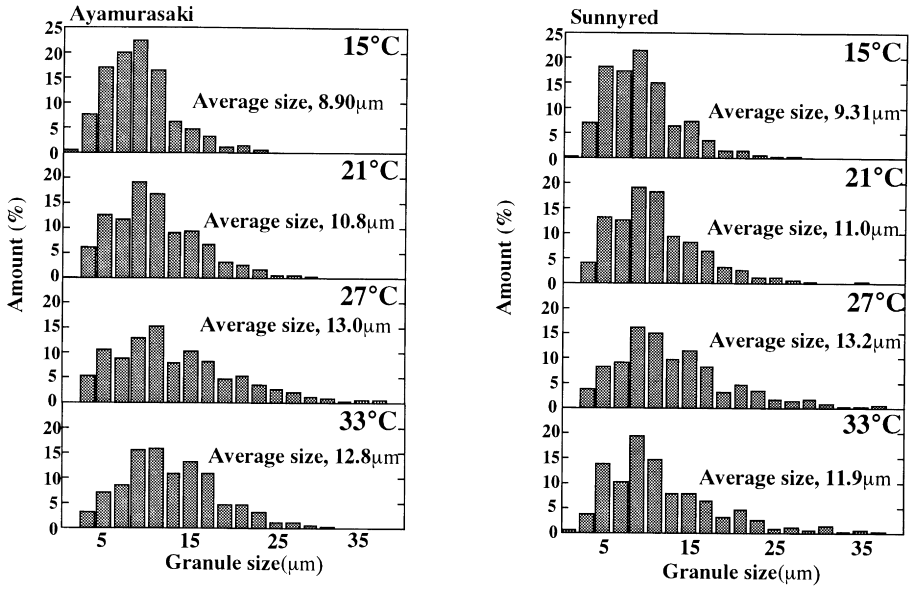

Fig. 1. Size distributions of starch granules from sweet potatoes grown at four different soil temperatures.

in Fig. 1. The dominant granule size was 8–10 μm in most cases. The starches from the samples grown at 15°C had a narrower range of size distributions, from 2 to 26 μm. In contrast, at higher temperatures, granule size ranged widely. For example, the proportions of large-sized granules of more than 26 μm were 0, 0.9, 5.1 and 1.9% in Ayamurasaki grown at 15, 21, 27 and 33°C, respectively. They were 0.3, 1.4, 5.6 and 4.1% in Sunnyred grown at 15, 21, 27 and 33°C, respectively. The average granule size varied within the range of 8.90 to 13.0 μm and 9.31 to 13.2 μm for Ayamurasaki and Sunnyred, respectively. A large increase in the average granule size (Ayamurasaki, 4.10 μm; Sunnyred, 3.89 μm) was observed as the soil temperature rose from 15 to 27°C. On the other hand, less change in average granule size was seen when the soil temperature rose from 27 to 33°C.

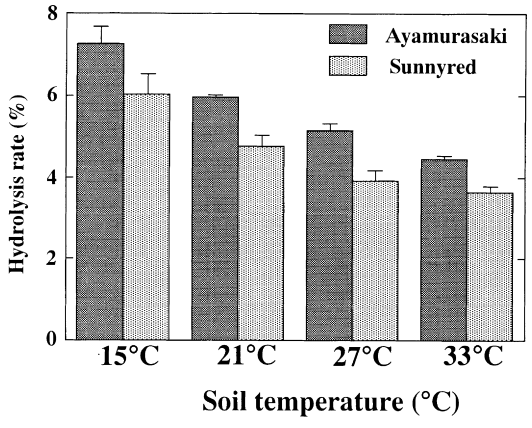

Fig. 2. Enzymatic digestibility of starch granules from sweet potatoes grown at four different soil temperatures. Results are expressed as means ± standard errors of three determinations.

Digestion of starch granules by crystalline glucoamylase of *Rhizopus niveus*, which has a high affinity to raw starch, was tested, and the results are shown in Fig. 2. The hydrolysis rate of starch granules from Ayamurasaki decreased from 7.25 to 4.45%, and those from Sunnyred decreased from 6.04 to 3.63% as the soil temperature rose from 15 to 33°C.

To study the fine structure of amylopectin, we examined the linear maltosaccharides released after starch debranching by isoamylase using HPAEC-PAD. Maltosaccharides with $DP < 6$ were scarcely detected in all cases. Area peaks between DP 6 and 45 were added, and the areas of individual peaks were divided by this sum. Chain length distributions (DP 6-45) of amylopectin calculated by this method are shown in Fig. 3. In both cultivars, amylopectins from the samples grown at 15 and 21°C peaked at DP 12, while those grown at 27 and 33°C peaked at DP 13. All amylopectins examined had a trough at DP 8, and the trough tended to be obscure at higher soil temperatures. Each chain was classified into three fractions (fa, DP 6-12; fb1, DP 13-24; fb2, DP 25-45). As suggested by Hanashiro, Abe and Hizukuri (1996), fractions fa, fb1 and fb2 probably correspond to A, B_1 and B_2 or longer chains, respectively. As proposed by Hizukuri (1986), A chains carry no branch chain and B chains carry one or more branch chains. In addition, A and B_1 chains are localized in one cluster, and B_2 chains span two clusters. The proportions of these fractions (fa, fb1 and fb2) are shown in Fig. 4. In most cases, amylopectins from the samples grown at 15°C had higher percentages of fa fractions and lower percentages of fb1 and fb2 fractions compared with those grown at higher temperatures. The percentages of fa decreased slightly as the soil temperature rose from 15 to 21°C, and to a greater extent as the treatment temperature went up to 33°C. The percentages

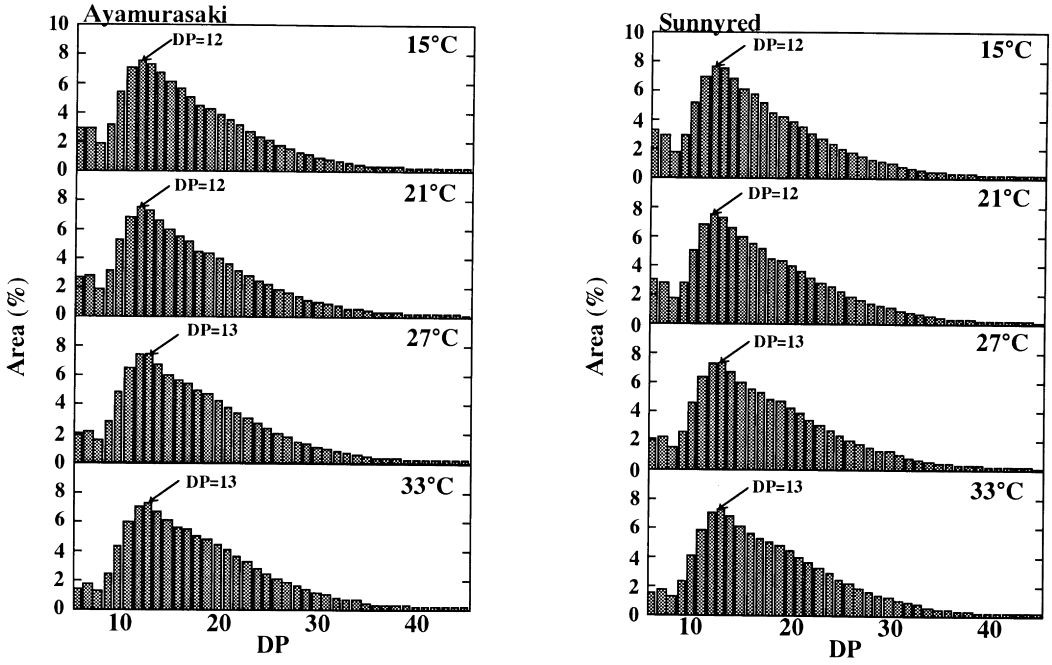

Fig. 3. Comparison of the chain length distributions of amylopectins from sweet potatoes at four different soil temperatures. Results are expressed as means of two determinations.

of fb1 and fb2 exhibited the opposite tendency to that of fa. As the values of the relative PAD response of malto-oligosaccharides between DP 6 and 17 had been determined by Koizumi et al. (1991), we used these values in calculating the molar distributions of the amylopectin chain length (DP 6-17). As can be seen in Fig. 5, lower soil temperatures led to a higher molar percentage of chains with DP 6-10 and a lower molar percentage of chains with DP 12-17. In particular, we found marked decreases in the contents of extremely short chains of DP 6 and 7 when the soil temperature rose from 15 to 33°C.

The thermal properties were analyzed by DSC, and the results are presented in Table 2. Soil temperature was found to have a large effect on the values of both T_o and T_p. When comparing the starches from the samples grown at 15°C with those grown at 33°C, the T_o of Ayamurasaki and Sunnyred increased by 25.8 and 21.8°C, respectively. Similarly, large increase in T_p (Ayamurasaki, 22.8°C; Sunnyred, 22.3°C) was detected as the soil temperature rose from 15 to 33°C. The values of ΔH varied from 12.2 to 17.6 J/g and from 13.0 to 16.3 J/g for Ayamurasaki and Sunnyred, respectively, exhibiting distinct differences as the soil temperature varied. Increasing soil temperatures caused higher values of ΔH.

We examined the pasting properties of starches by RVA, which measures the viscosity during and after gelatinization.

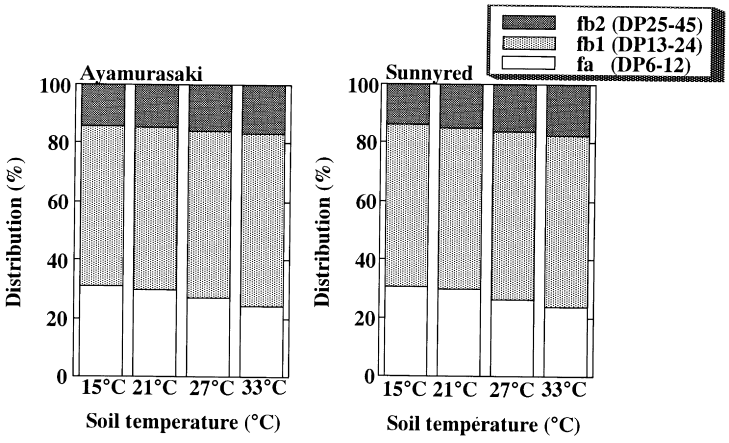

Fig. 4. Percentages of fa, fb1 and fb2 of amylopectins from sweet potatoes grown at four different soil temperatures. Results are expressed as means of two determinations.

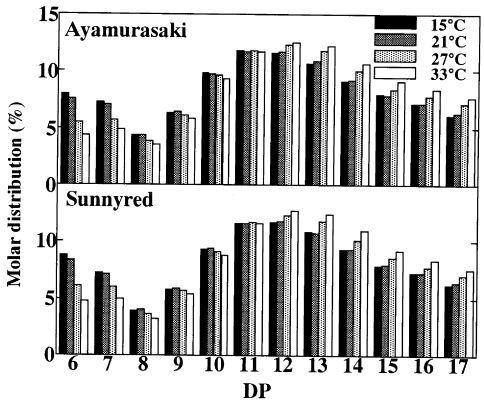

Fig. 5. Molar distributions (% of total) of unit-chains between DP 6 and 17 of amylopectins from sweet potatoes grown at four different soil temperatures. Results are expressed as means of two determinations.

Fig. 6 shows the RVA curve of all the starches examined, and also defines the peak viscosity, breakdown and setback. The RVA results are also presented in Fig. 7. Peak viscosity ranged from 282 to 382RVU and from 265 to 369RVU for Ayamurasaki and Sunnyred, respectively. A clear increase in peak viscosity was observed with the elevation of the soil temperature from 15 to 27°C. However, the starches from the sample grown at 33°C did not reveal higher peak viscosities compared with those grown at 27°C. The breakdown of Ayamurasaki and Sunnyred varied within the range of 113–166RVU and 108–166RVU, respectively. A distinct enhancement in breakdown was found as the soil temperature rose from 15 to 27°C. On the other hand, sweet potatoes grown at 33°C produced starch with a lower breakdown than those grown at 27°C. The values of setback ranged from 53 to 96RVU and from 59 to 89RVU for Ayamurasaki and Sunnyred, respectively. A high soil temperature generally tended to reduce the values of setback. The distributions of pasting temperatures of the Ayamurasaki and Sunnyred starches were found to be, respectively, 65.3–82.4°C and 65.3–81.6°C. A high soil temperature led to a clear enhancement of the pasting temperature.

4. Discussion

The results presented here demonstrate the contribution of soil temperature during sweet potato tubers development on the physicochemical properties of starch, using samples grown in a greenhouse in which temperatures of air and soil were controlled independently. Amylose content, granule size, enzymatic digestibility, gelatinization and pasting properties, as well as the fine structure of amylopectin, were affected greatly by soil temperature.

Information on the relationship between amylose content and environmental temperature during plant development has been accumulated to some extent. According to Asaoka et al. (1985), starches from rice plants grown at 30°C had a lower amylose content than those grown at 25°C. Similarly,

Table 2
Gelatinization properties by DSC of starches from sweet potatoes grown at four different soil temperatures (values are means of three determinations)

Cultivar	Soil temperarture (°C)	T_o (°C)[a]	T_p (°C)[a]	ΔH (J/g)[b]
Ayamkurasaki	15	51.2	56.7	12.2
	21	59.1	64.6	15.1
	27	71.4	74.2	16.4
	33	76.2	79.5	17.6
Sunnyred	15	52.3	56.6	13.0
	21	58.3	63.6	14.9
	27	68.9	72.8	15.4
	33	74.3	78.9	16.3

[a] Standard deviation ±0.3°C.
[b] Standard deviation ±0.6 J/g.

Lu et al. (1996) reported that the amylose content of starches from two maize varieties decreased as the developmental temperature increased from 25 to 35°C. In contrast, higher growth temperatures tended to lead to an increase in the amylose content of wheat starches (Shi et al., 1994; Tester et al., 1995). In barely, amylose content was not dramatically altered by growth temperature (Tester et al., 1991). Potato starch had an almost constant amylose content, even when the environmental temperature was raised from 10 to 25°C (Hizukuri, 1969). In this study, a low amylose content of starches was observed in sweet potatoes grown at lower soil temperatures. These results indicate that the influence of developmental temperature on amylose synthesis varies with plant species. It is well known that amylose synthesis is controlled by a granule-bound isoform of starch synthase, known as *Waxy* (*Wx*) protein. According to the report of Sano, Maekawa and Kikuchi (1985), in rice, not only amylose content but also the amount of *Wx* protein increased at a cool temperature (21°C), compared with the values obtained in plants grown at a normal temperature (27°C). Additionally, Hirano and Sano (1998) recently demonstrated that the expression of the rice *Wx* gene was enhanced in response to a cool temperature (18°C). It is suggested that the expression of the sweet potato *Wx* gene is activated at a higher temperature than is the case for rice. We previously observed a relatively small increase in amylose content (ca. 2.0%) from two sweet potato varieties with earlier planting and harvesting times (Noda et al., 1997). The soil temperature range of our previous study was presumed to be too narrow to have a large effect on amylose content.

It has been documented that in some plant species, environmental temperature affects the size distributions of starch granules. In an early study (Hizukuri, 1969), it was reported that a higher environmental temperature resulted in a slight decrease in average starch granule size in potatoes. Lu et al. (1996) and Shi et al. (1994) reported the effect of environmental temperature on the size of wheat and maize starch granules, respectively. In both cases, starch granule sizes decreased as the environmental temperature was

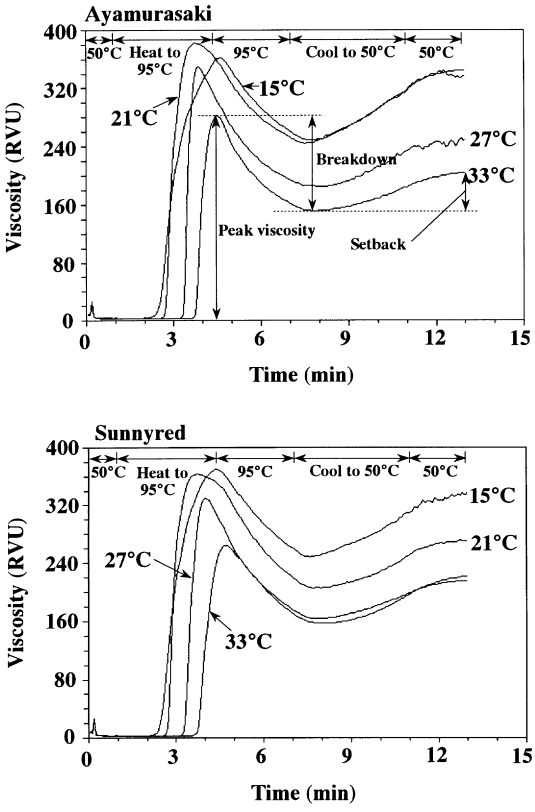

Fig. 6. RVA viscograms of starches from sweet potatoes grown at four different soil temperatures.

raised. Contrary to these findings, raising the soil temperature from 15 to 27°C led to an apparent increase in average granule size in this investigation. The reason for these phenomena is unknown because the precise mechanism for determining the size of starch granules is not well understood.

A clear decrease in the hydrolysis rate of starch granules by crystalline glucoamylase of *Rhizopus niveus* was found with the elevation of soil temperature. The result was in agreement with the report of Hizukuri (1969), who showed the amylase susceptibility of raw starch granules prepared from sweet potatoes grown at higher soil temperatures. One of the main factors in the digestibility of raw starch by amylase has been thought to be the size of starch granules. In our previous investigation, using 30 kinds of sweet potato starches, significant negative correlations were obtained between the average size of starch granules and the digestibility of each one of two glucoamylases (Noda, Takahata & Nagata, 1993). Such a trend could be reconfirmed in this study since a higher soil temperature was likely to enhance the size of starch granules and reduce digestibility by glucoamylase.

In general, it was shown that the distributions of amylopectin chain length were sensitive to environmental temperatures. In rice, gel permeation chromatography (Asaoka et al., 1985) and HPAEC (Umemoto, Nakamura, Sato & Terashima, 1999) revealed a reduction in short chains of amylopectin with the elevation of environmental temperature. Lu et al. (1996) reported the effect of growing temperature on amylopectin structure in two maize varieties, ICI63 and ICI92. They showed that as the developmental

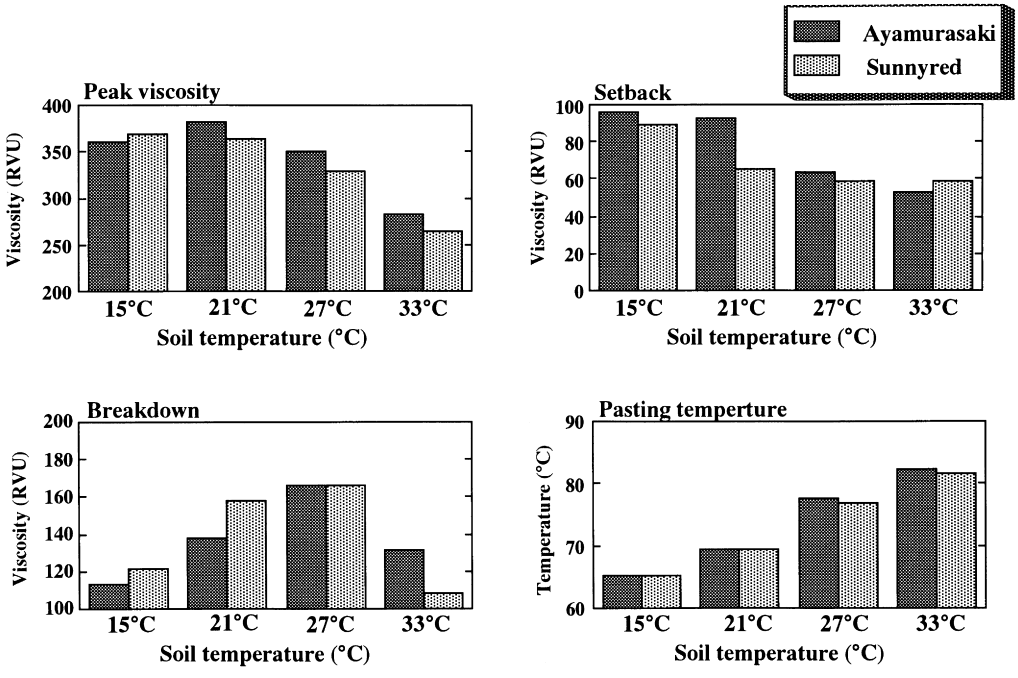

Fig. 7. Pasting properties by RVA of starches from sweet potatoes grown at four different soil temperatures. Results are expressed as means of two determinations.

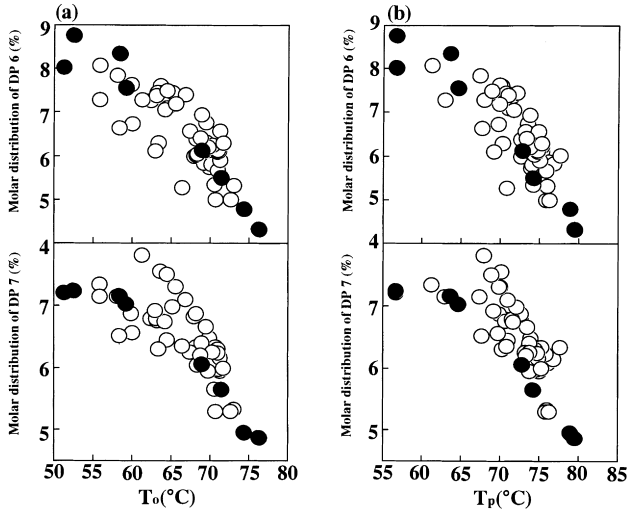

Fig. 8. Relationships between either (a) T_o or (b) T_p and either the molar percent of unit-chain with DP 6 or that with DP 7 in starch samples from 59 kinds of sweet potato starches. ○ and ● indicate 51 kinds of samples described in our previous study (Noda et al., 1998b) and 8 kinds of samples used in this study, respectively.

temperature was raised from 25 to 35°C, ICI63 amylopectin had an increased medium branch-chain fraction and had decreased long and short-chain fractions, whereas ICI92 had increased long and medium branch-chain fractions and a decreased short branch-chain fraction. In wheat, an elevated environmental temperature increased the number of chains with DP 10-16 but reduced the number of chains with DP 17-21 (Shi et al., 1994). However, exceptional data was found, using gel permeation chromatography, that indicated there was little influence of growth temperature on the amylopectin structure obtained in barley (Tester et al., 1991). In this study, the distributions of amylopectin chain length observed by HPAEC were affected by soil temperature during sweet potato development. Higher soil temperatures caused an apparently lower number of fa fractions (DP 6-12) and higher numbers of fb1 (DP 13-24) and fb2 (DP 25-45) fractions. Thus, the elevation of environmental temperatures led to a reduction in short chains of amylopectin except in the cases of wheat and barley. Amylopectin is believed to be formed mainly due to the coordinated action of branching enzyme and soluble starch synthase. The former is capable of hydrolyzing an α-1,4 bond of a glucan chain and linking the separated chain segment to an acceptor chain via an α-1,6 bond. Multiple forms of the branching enzyme, BE I and BE II, have been found in maize. It was reported that BE II preferentially transferred short chains and had a lower optimum temperature compared with BE I (Guan, Li, Imparl-Radosevich, Preiss & Keeling, 1997; Takeda, Guan & Preiss, 1993). On the basis of these results, in maize grown at a higher temperature, the decrease in the ratio of BE II to BE I may lead to a reduction in short chains of amylopectin, as suggested by Lu et al. (1996). Although sweet potato roots contain 7 isoforms of branching enzyme, the characteristics of these isozymes have not been examined (Nakayama & Nakamura, 1994). For this reason, the precise mechanism explaining temperature-dependent variations in amylopectin structure is not well clarified for the sweet potato.

With regard to the effects of soil temperature on gelatinization properties determined by the DSC of sweet potato starches, there are marked effects on the gelatinization temperatures (T_o and T_p). The elevation of soil temperatures resulted in increases of both T_o and T_p by at least 20°C. An increase in gelatinization temperatures with increasing environmental temperatures was also found in barley (Tester et al., 1991), wheat (Shi et al., 1994; Tester et al., 1995) and maize (Lu et al., 1996). However, the ranges in T_o and T_p among environmental temperatures were narrower in these studies. A higher soil temperature was definitely associated with higher values of ΔH in sweet potato starches. This is not in agreement with previous studies on barley (Tester et al., 1991) and wheat (Shi et al., 1994; Tester et al., 1995), where ΔH remained constant or rose slightly as environmental temperatures increased.

Our previous research, using 51 kinds of sweet potato starches, showed that the quantitative molar distributions of amylopectin chain length (DP 6-17) analyzed by HPAEC had a great impact on all the DSC parameters, T_o, T_p and ΔH, whereas amylose content was found to be independent of all the DSC parameters (Noda et al., 1998b). Increases in short unit-chains with DP 6-10 resulted in lower T_o, T_p and ΔH in general. The contributions of the number of extremely short unit-chains with DP 6 and 7 to T_o and T_p were particularly large. In contrast, each proportion of the unit-chains with DP 12-17 was positively correlated to T_o, T_p and ΔH. In a composite of 51 kinds of samples described in our previous study and 8 kinds of samples used in this study, we attempted to calculate the correlation between starch gelatinization temperatures (T_o and T_p) and each number of unit-chains with DP 6 and 7. As shown in Fig. 8., the correlations of T_o to the molar percentage of a unit-chain with DP 6 and that with DP 7 were significant and negative (DP 6, $r = -0.833$, $p < 0.01$; DP 7, $r = -0.766$, $p < 0.01$). T_p was also negatively correlated with the molar percentage of a unit-chain with DP 6 ($r = -0.813$, $p < 0.01$) and that with DP 7 ($r = -0.742$, $p < 0.01$). Our results to date strongly suggest that starch with a lower gelatinization temperature has abundant, extremely short unit-chains with DP 6 and 7 of amylopectin molecules within the same botanical origin for sweet potatoes.

Starch pasting properties are thought to be important predictors of food products such as noodles. Compared to some other properties of starch, there has not been a great deal of information concerning the relationships between environmental temperature during plant development and starch pasting properties. According to the early report of Hizukuri (1969), who studied the effect of environmental temperature on starch pasting properties using amylograph in rice, potatoes, soybean seedlings and sweet potatoes, the

pasting temperature rose as the environmental temperature rose in all cases. Furthermore, with the elevation of the environmental temperature, the peak viscosity fell in potatoes and sweet potatoes, while it rose in rice and remained almost constant in soybean seedlings. In this investigation, higher soil temperatures during sweet potato development caused higher pasting temperatures, as found in T_o and T_p by DSC. As soil temperatures rose from 15 to 27°C, peak viscosity and breakdown decreased clearly. In addition, higher soil temperatures tended to reduce setback. We could not compare the present data for breakdown and setback to the previous data presented by Hizukuri (1969) because he did not provide the values for breakdown and setback. However, the present data for the pasting temperature and peak viscosity were in line with the previous data, as mentioned above.

The present investigation indicates that a major difference in starch properties, especially gelatinization temperature and amylopectin structure, occurs according to soil temperature during the development of sweet potato tuberous roots. The precise mechanism explaining such temperature-dependent variations in starch properties remains to be elucidated. Our investigation would offer useful information for breeders, farmers and starch users.

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