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Physicochemical properties of amylose-free starch from transgenic sweet potato

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Abstract

A transgenic amylose-free sweet potato has been obtained by introduction of granule-bound starch synthase I (GBSSI) cDNA of sweet potato in sense orientation (Plant Cell Reports (2001)). In this study, starches from 6 transgenic sweet potatoes produced by introduction of GBSSI cDNA, including the amylose-free transformant, were analyzed for their physicochemical properties, granule-size distribution, enzymatic digestibility, amylopectin structure, gelatinization properties and pasting properties. We observed little difference in granule size distribution between amylose-free starch and control starch. Amylose-free starch was more susceptible to glucoamylase digestion than control starch. The amylopectin of the amylose-free transformant was found to have a slightly lower content of short chains. Amylose-free starch showed higher gelatinization temperature and gelatinization enthalpy and lower setback in comparison with control starch. Thus, it was found that the starch from the amylose-free transgenic sweet potato possessed unique physicochemical properties.

Keywords: Amylose-free starch; Physicochemical properties; Gelatinization temperature

1. Introduction

As the use of sweet potato starch in Japan is comparatively limited, it is of interest to develop varieties of sweet potato that contain starch with unique physicochemical characteristics. Amylose and amylopectin are the two main polysaccharide components of starch. The textural properties of starch are altered according to the large variation in the amylose–amylopectin ratio. In sweet potato, relatively narrow ranges in amylose content (10–25%) have been observed in comparison with other crops (Noda et al., 1998b). Therefore, it is necessary to expand the range of amylose content to provide sweet potato starch with the desired textural properties. It has been accepted that alteration in amylose content by genetic engineering contributes to the improvement of starch quality. It has been established that amylose synthesis in storage starch granules is controlled by granule-bound starch synthase I (GBSSI), which is known as the Wx protein. It was reported that low-amylose starch was obtainable by the antisense GBSSI RNA technique in potato (Kuipers, Jacobsen, & Visser, 1994; Visser et al., 1991) and rice (Shimada, Tada, Kawasaki, & Fujita, 1993). Recently, Kimura, Ideta, and Saito (2000) succeeded in isolating full-length cDNA for sweet potato GBSSI. Furthermore, 26 transgenic sweet potato plants were produced by introduction of the GBSSI cDNA of sweet potato in sense orientation (Kimura et al., 2001). After analyzing the amylose content of the starches from these transgenic sweet potatoes, one transgenic plant was found to contain no amylose (Kimura et al., 2001). This is the first demonstration of the production of an amylose-free transformant of sweet potato.

In this paper, we report on the physicochemical properties of starches of such sweet potato transgenic plants, including the amylose-free transformant. The effect of an extremely decreased amylose content on the physicochemical properties of sweet potato starch is described.

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Table 1
The yield and apparent amylose content of starches from transgenic sweet potatoes

Sample[a]	Starch yield (%)[b]	Apparent amylose content (%)[c]
C	20.9	18.5
1 (Amylose-free)	14.3	0[d]
2	17.5	19.0
3	20.9	18.9
4	19.4	19.5
5	20.3	19.4
6	20.7	19.2

[a] C, untransformed control plant; 1–6, transgenic plants (1, amylose-free transformant).
[b] Dry weight basis. Values are the results of one determination.
[c] Values are means of three determinations. SD ± 0.7%.
[d] The BVs of sweet potato (Koganesengan) amylose and amylopectin reported by Takeda et al. (1986) were 1.48 and 0.166, respectively. On the other hand, that of transgenic plant No. 1 was 0.141. Therefore, the apparent amylose content calculated from BVs of sweet potato (Koganesengan) amylose and amylopectin was lower than 0% for transgenic plant No. 1.

2. Materials and methods

2.1. Starch samples

Twenty-six sweet potato transformants introduced with GBSSI cDNA in sense orientation have been obtained as described by Kimura et al. (2001). Out of 26 transgenic plants, 6 plants named transgenic plant No. 1, No. 2, No. 3, No. 4, No. 5 and No. 6 were used in this study. The roots of transgenic plant No. 1 had amylose-free starch, while roots of other transformants had normal starch (Kimura et al., 2001). Starch granules were isolated from each sample root as reported earlier (Noda, Takahata, Nagata, & Monma, 1992a). Starches from maize and wheat were obtained from Wako Pure Chemical Co., Osaka, Japan.

2.2. Analytical methods

Apparent amylose content was determined from the blue value (BV) at 680 nm, using defatted starch, not intact starch (Noda et al., 1992a). In this calculation, we used the BVs of sweet potato (Koganesengan) amylose and amylopectin for standards as described by Takeda, Tokunaga, Takeda, and Hizukuri (1986). 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 et al., 1992a). The enzymatic digestibility of the raw starch by crystalline glucoamylase of *Rhizopus niveus* was performed for 4 h at 40 °C as described earlier (Noda, Takahata, & Nagata, 1992b). The reaction mixtures (1 ml) contained 2% substrate, 5 units of glucoamylase and 25 mM acetate buffer (pH 5.0). One unit of glucoamylase was defined as the amount of enzyme that liberated 1 μmol of reducing sugar (as glucose) per min from soluble starch at 40 °C at pH 5.0. Each pasted starch, which was prepared by suspending in alkaline solution, was digested by *Pseudomonas amyloderamosa* isoamylase, as described earlier (Noda, Takahata, &

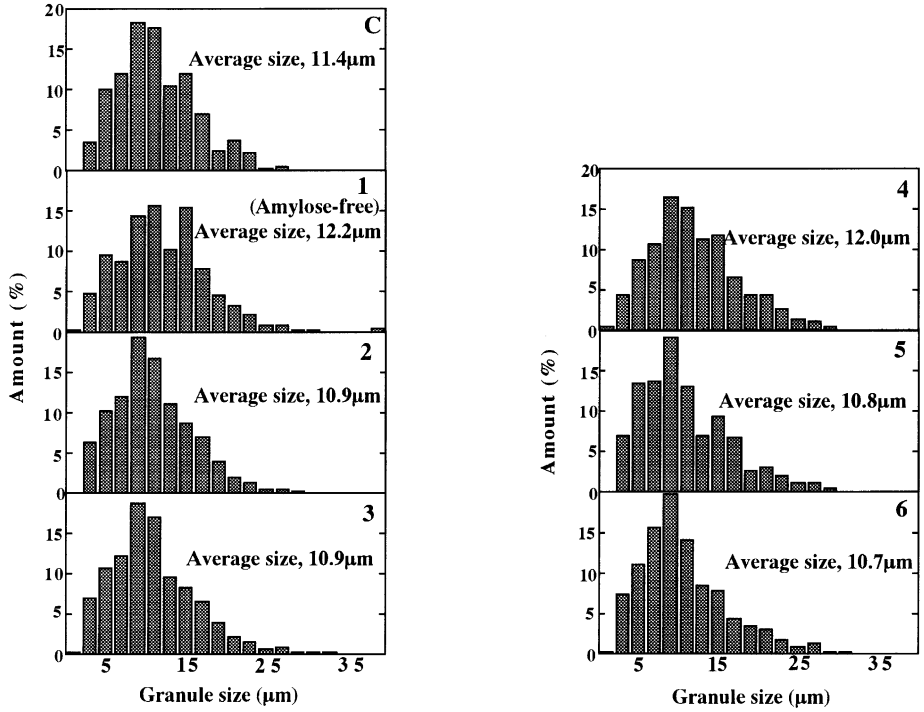

Fig. 1. Size distributions of starch granules from transgenic sweet potatoes compared with non-transgenic sweet potato. C, untransformed control plant; 1–6, transgenic plants (1, amylose-free transformant).

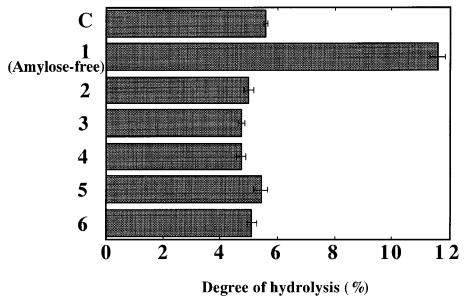

Fig. 2. Enzymatic digestibility of starch granules from transgenic sweet potatoes compared with non-transgenic sweet potato. C, untransformed control plant; 1–6, transgenic plants (1, amylose-free transformant). Results are expressed as means of three determinations.

Sato, 1995). The linear maltosaccharides produced after enzyme digestion 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 reported by Koizumi, Fukuda, and Hizukuri (1991). The area of each peak of linear chains up to DP 35 was determined using a Hitachi D-2500 Chromato-integrator. Differential scanning calorimetry (DSC) measurements were conducted 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). Rapid visco-analyzer (RVA) measurements were made using the RVA-3D (Newport Scientific Pvt. Ltd., Australia) with a starch concentration of 8% as described previously (Noda, Kobayashi, & Suda, 2001). The viscosity was measured in RVA units.

3. Results

As shown in Table 1, the apparent amylose content determined by the BV at 680 nm was 0% (the calculated value was less than 0%) for transgenic plant No. 1, which corresponds to the amylose-free transformant. The range of apparent amylose content was 18.5–19.5% for the untransformed control and the other five transformants. Compared to our values, the slightly lower apparent amylose content values obtained by Kimura et al. (2001) may be due to the absence of starch defatting in their study.

Examination of starch granules by light microscopy indicated that all starches had spherical shapes, which is typical of sweet potato starches (data not shown). The distributions of starch granule size determined by an image analyzer attached to a light microscope are presented in Fig. 1. The dominant granule size was 8–10 μm in all cases. The average granule size ranged from 10.7 to 12.2 μm. These values were in good agreement with our previous research on 12 sweet potato starches (Noda et al., 1992a). Thus, there were negligible differences in granule shape and size regardless of the presence or absence of amylose.

To determine amylase digestibility, each starch was treated with crystalline glucoamylase from *Rhizopus niveus*, which possesses a high affinity to raw starch (Takaya, Glover, Sugimoto, Tanaka, & Fuwa, 1982). It can clearly be seen from Fig. 2 that amylose-free starch had about

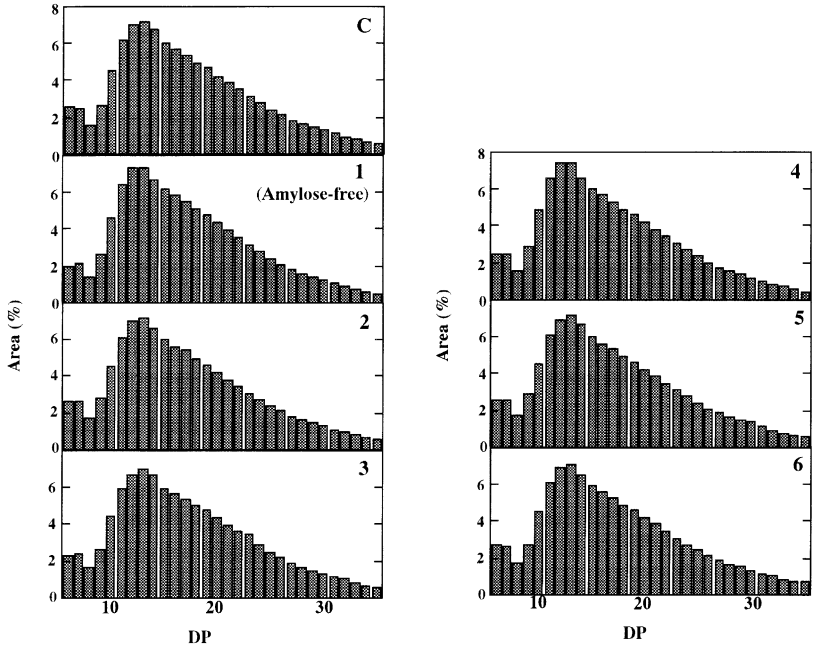

Fig. 3. Chain-length distributions of amylopectins from transgenic sweet potatoes compared with non-transgenic sweet potato. C, untransformed control plant; 1–6, transgenic plants (1, amylose-free transformant). Results are expressed as means of two determinations.

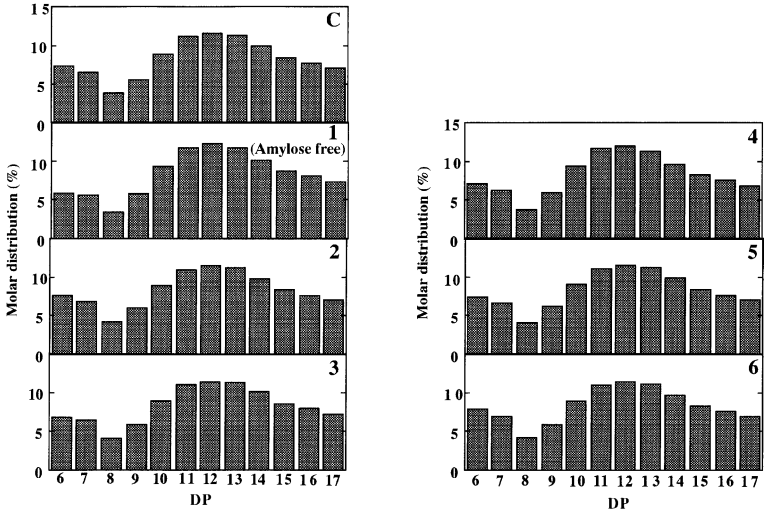

Fig. 4. Molar distributions (% of total) of unit-chains between DP 6 and 17 of amylopectins from transgenic sweet potatoes compared with non-transgenic sweet potato. C, untransformed control plant; 1–6, transgenic plants (1, amylose-free transformant). Results are expressed as means of two determinations.

two-fold higher degree of hydrolysis than the control native sweet potato starch. In addition, amylose-free starch was more susceptible to glucoamylase digestion than starches from maize and wheat, which have higher digestibility by amylase than sweet potato starch in general. The relative degrees of hydrolysis for the amylose-free transformant, wheat, maize and the control starch were 1.00, 0.94, 0.82 and 0.48, respectively (data not shown). In contrast, starches from the other sweet potato transformants showed almost the same hydrolysis rate by glucoamylase as the control starch.

The isoamylase-treated starch was loaded on to a HPAEC column to determine the distributions of amylopectin chain length. Based on the peak area, chain-length distributions (DP 6–35) were calculated. The results are shown in Fig. 3. In addition, using the values of the relative PAD response of malto-oligosaccharides between DP 6 and 17 (Koizumi et al., 1991), the molar distributions of amylopectin chains were determined and the results are presented in Fig. 4. All amylopectins had a trough at DP 8, which is in agreement with the report of Koizumi et al. (1991). Differences between the amylose-free transformant and the control could be seen in extremely short chains of DP 6–8. The amylose-free transformant had a decreased content of DP 6–8 in amylopectin molecules in comparison with the control. The patterns of amylopectin chain length for the other transformants were the same as those for the control except that transgenic plant No. 3 contained slightly fewer chains of DP 6–8 than the control.

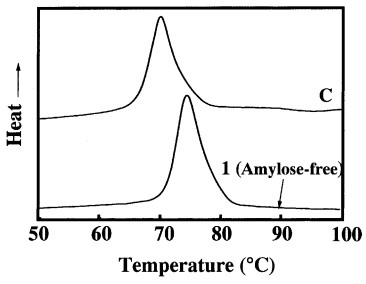

Fig. 5. DSC thermograms of starches from non-transgenic sweet potato (C) and transgenic amylose-free sweet potato (1).

Fig. 5 shows the starch gelatinization curves of the control and the amylose-free transformant by DSC. As can be seen, DSC curves varied between the control and the amylose-free transformant. The peak of the gelatinization curve was larger and shifted to a higher temperature for the amylose-free transformant. The results of the DSC parameters are shown in Table 2. Clear differences in all DSC parameters, T_o, T_p, and ΔH were observed among the starch samples examined. The highest T_o (71.3 °C), T_p (74.6 °C) and ΔH (17.2 J/g) values were found for the amylose-free transformant. Transgenic plant No. 3, which had a slightly reduced content of amylopectin short chains, tended to have higher values of T_o (69.5 °C), T_p (72.8 °C) and ΔH (15.5 J/g). For the other four transformants, the values of T_o, T_p, and ΔH were similar to those for the control.

Table 2
Gelatinization properties by DSC of starches from transgenic sweet potatoes (values are means of three determinations)

Sample[a]	T_o (°C)[b]	T_p (°C)[b]	ΔH (J/g)[c]
C	66.4	69.9	14.8
1 (Amylose-free)	71.3	74.6	17.2
2	67.9	71.1	15.5
3	69.5	72.8	15.5
4	68.4	72.1	15.0
5	67.3	71.0	14.9
6	66.2	69.5	14.5

[a] C, untransformed control plant; 1–6, transgenic plants (1, amylose-free transformant).
[b] SD ± 0.3 °C.
[c] SD ± 0.5 J/g.

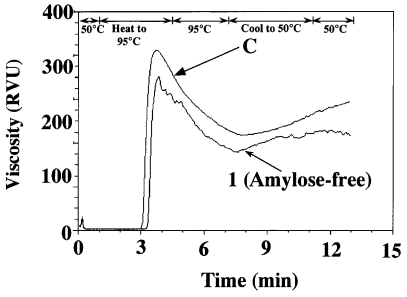

Fig. 6. RVA viscograms of starches from non-transgenic sweet potato (C) and transgenic amylose-free sweet potato (1).

The starch pasting curves by RVA of the control and the amylose-free transformant are shown in Fig. 6. An irregular line was observed for the RVA curve of the amylose-free transformant. Kortstee et al. (1998) also reported that the RVA curve was irregular for amylose-free potato starch. They presumed that this phenomenon was caused by the higher sensitivity of amylose-free starch granules to shear. Table 3 shows the pasting parameters by RVA. Peak viscosity varied from 275 to 330 RVU, displaying the lowest value for the amylose-free transformant. Breakdown varied from 124 to 154 RVU. The amylose-free starch was at neither extreme, but closer to the low end. A much lower value of setback was detected for amylose-free transformant. As expected, the amylose-free transformant appeared to contain starch that is difficult to retrograde. The pasting temperature was the highest for the amylose-free transformant (77.6 °C) and the second highest for transgenic plant No. 3 (75.9 °C).

4. Discussion

In this paper, we have described the physicochemical properties of starch in sweet potatoes among which the transgenic plants of an amylose-free transformant are included. Enzymatic digestibility and pasting properties by RVA were dramatically affected by a vast reduction of amylose content. It has been documented that starch granules from waxy maize (Fuwa, Nakajima, Hamada, & Glover, 1977), waxy rice (Evers & Juliano, 1976), and waxy barley (Fuwa, 1982) are digested by amylase faster than their normal counterparts in general. In this study, starch from an amylose-free sweet potato transformant was also much more susceptible than the control starch to degradation by the crystalline glucoamylase of *Rhizopus niveus*. We have examined the factors involved in the digestibility of raw starch by amylase. In our previous report (Noda, Takahata, & Nagata, 1993), using 30 kinds of sweet potato starches, average granule size was negatively correlated with digestibility by glucoamylase, while there was not a significant correlation between apparent amylose content and digestibility. However, as the range of apparent amylose content, we had observed was relatively narrow (18.2–23.7%), further investigation was required to clarify the contribution of apparent amylose content to digestibility by amylase. In our more recent studies (Noda et al., 1996, 2001; Noda, Takahata, Sato, Ikoma, & Mochida, 1997), average granule size, apparent amylose content and digestibility by the glucoamylase of *Rhizopus niveus* were also determined, using a total of 24 sweet potato starch samples. In the composite of 54 kinds of sweet potato starches described in our previous studies (Noda et al., 1993, 1996, 1997, 2001) and seven kinds of sweet potato starches used in this study, correlations were recalculated between digestibility and average granule size as well as between digestibility and apparent amylose content. As shown in Fig. 7, a highly negative significant correlation ($r = -0.640$, $P < 0.01$) was observed between digestibility and apparent amylose content. In contrast, a slightly negative significant correlation was detected between average granule size and digestibility ($r = -0.303$, $P < 0.05$). When amylose-free starch was excluded, the correlation coefficients between digestibility and apparent amylose content and those between digestibility and average granule size were -0.325 ($P < 0.05$) and -0.454 ($P < 0.01$), respectively. These results indicate that the digestibility of raw starch by amylase is influenced more by a marked decrease in apparent amylose content than by variation in the average granule size.

It has been reported that GBSSI plays a role in the synthesis of amylopectin as well as amylose (Van de Wal et al., 1998). However, there has been limited information on the influence of the inhibition of GBSSI on the molecular structure of amylopectin. Although transgenic potato (Kuipers et al., 1994; Visser et al., 1991) and rice (Shimada et al., 1993)

Table 3
Pasting properties by RVA of starches from transgenic sweet potatoes (values are means of two determinations)

Sample[a]	Peak viscosity (RVU)	Breakdown (RVU)	Setback (RVU)	Pasting temperature (°C)
C	327	149	61	74.3
1 (Amylose-free)	275	133	32	77.6
2	296	124	68	74.3
3	308	145	50	75.9
4	322	138	60	75.2
5	330	154	61	74.3
6	323	128	64	73.5

[a] C, untransformed control plant; 1–6, transgenic plants (1, amylose-free transformant).

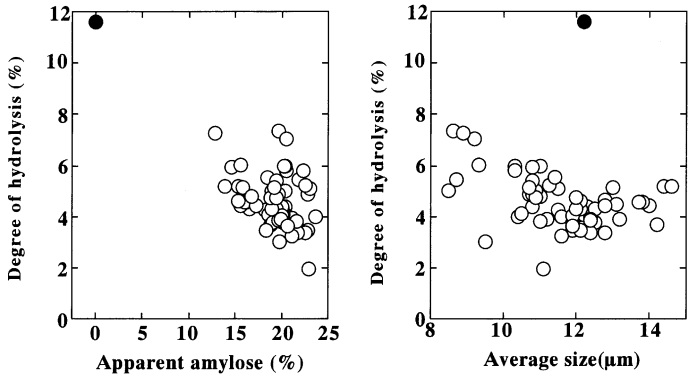

Fig. 7. Relationships between digestibility and average granule size as well as between digestibility and apparent amylose content in starch samples from 54 kinds of sweet potatoes. ● indicates starch from transgenic amylose-free sweet potato.

mutants in which the expression of GBSSI is inhibited have been described, the molecular characteristics of their amylopectins have not been determined. Our present HPAEC results indicate that the contents of the short chains were slightly lower in the amylose-free transformant than in the control. In *Chlamydomonas reinhardtii*, the amylose-defective mutant has been shown to accumulate a structurally modified amylopectin (Delrue et al., 1992). For the case of non-transgenic crops, Yasui, Matsui, Sasaki, and Yamamori (1996) have demonstrated, using the HPAEC technique, that the amylopectin structure of the waxy starch isolated from waxy wheat lines is essentially identical to that of the non-waxy starch from their non-waxy parents. To reach a clear conclusion regarding the relationship between the deficiency in GBSSI and amylopectin structure, further research would be needed.

Recently, we have proved that the variations in starch gelatinization properties measured by DSC are due to the molecular structure of amylopectin analyzed by HPAEC within the same botanical origin for sweet potato (Noda et al., 1998b, 2001). Starches with lower T_o, T_p and ΔH were shown to have higher content of extremely short chains with DP 6 and 7 in amylopectin molecules, while the amylose–amylopectin ratio did not have an impact on DSC parameters. However, when starches with extreme variations in amylose content were used, whether or not the amylopectin structure actually affected gelatinization properties remained to be answered. Several results have been obtained in which waxy starch tends to have higher values of T_p and ΔH in starch gelatinization than non-waxy starch (Fujita, Donghui, Sugimoto, Inouchi, & Fuwa, 1989; Fujita, Morita, & Fujiyama, 1993; Fujita, Yamamoto, Sugimoto, Morita, & Yamamori, 1998; Yasui et al., 1996). In the present study, we have analyzed the distributions of amylopectin chain length by HPAEC and gelatinization properties by DSC using seven kinds of sweet potato starches with a wide range of apparent amylose content (0–19.5%). To reconfirm the above theory, we investigated the relationships between DSC parameters (T_o, T_p, and ΔH) and the molar percentage of unit-chains with DP 6 and with DP 7

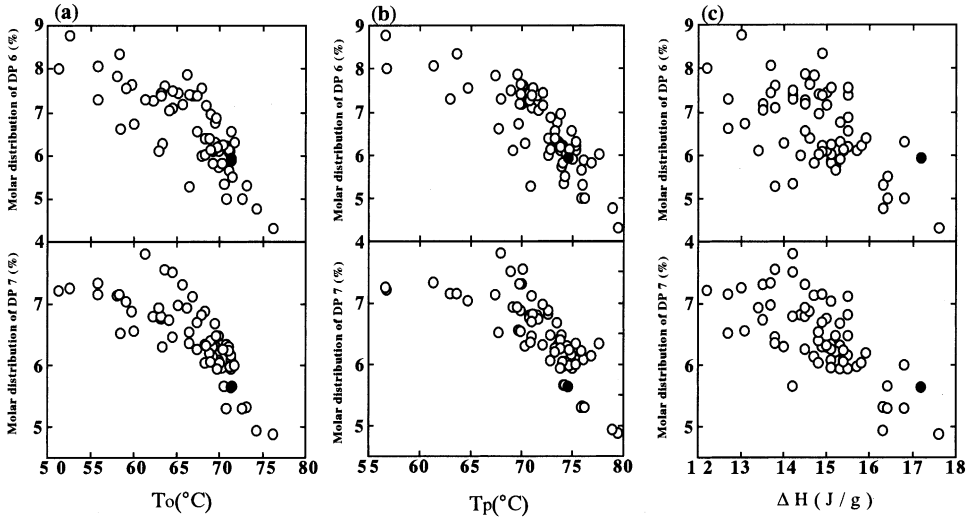

Fig. 8. Relationships between either (a) T_o, (b) T_p or (c) ΔH and either the molar percent of unit-chain with DP 6 or that with DP 7 in starch samples from 66 kinds of sweet potatoes. ● indicates starch from transgenic amylose-free sweet potato.

in all 66 kinds of sweet potato starches described in this (7 samples) and previous (59 samples) (Noda et al., 1998a,b, 2001) reports. As shown in Fig. 8, the molar percentage of DP 6 and that of DP 7 were negatively correlated with T_o (DP 6, $r = -0.784$, $P < 0.01$; DP 7, $r = -0.757$, $P < 0.01$), T_p (DP 6, $r = -0.797$, $P < 0.01$; DP 7, $r = -0.742$, $P < 0.01$) and ΔH (DP 6, $r = -0.574$, $P < 0.01$; DP 7, $r = -0.704$, $P < 0.01$) even when amylose-free starch was included. Our results with HPAEC analysis strongly suggested that the amylopectin molecular structure, rather than a significant reduction in apparent amylose content, has a large effect on starch gelatinization properties by DSC.

In Japan, most sweet potato starch is used in alcohol fermentation and the production of glucose and high-fructose syrups. In conventional enzymatic saccharification, starch is gelatinized by heating and liquefied with α-amylase at the same time. In recent years, the saccharification of raw starch by special fungi glucoamylase has been noted as an energy-saving technique (Abe, Bergmann, Obata, & Hizukuri, 1988). Thus, examining the digestibility of raw starch by glucoamylase is of importance using sweet potato starches. It has been known, however, that cereal starches are more easily digested than starches from root crops such as potato and sweet potato. On this account, it has been desired to screen varieties of sweet potato for ease of digestion by glucoamylase. The present study has revealed that the digestibility by crystalline *Rhizopus niveus* glucoamylase is markedly higher for amylose-free starch. The data on digestibility leads us to the conclusion that amylose-free sweet potato is a promising material for starch saccharification without cooking using a glucoamylase capable of digesting raw starch. The development of sweet potato cultivars that contain starch with unique physicochemical properties may allow use of the starch for other than saccharification. In particular, retrogradation is an important factor for starch used in food products. The setback of amylose-free starch paste was clearly lower, implying less retrogradation. Such unique characteristics, i.e. its higher digestibility by glucoamylase and lower setback, may be useful for special products within the food industry.

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