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Relationships between chain length distribution of amylopectin and gelatinization properties within the same botanical origin for sweet potato and buckwheat

Takahiro Noda[a,*], Yasuhiro Takahata[a], Tetsuo Sato[a], Ikuo Suda[a], Toshikazu Morishita[a], Koji Ishiguro[b], Osamu Yamakawa[b]

[a]*Department of Crop Breeding, Kyushu National Agricultural Experiment Station, MAFF, 2421 Suya, Nishigoshi, Kumamoto, 861-11 Japan*
[b]*Department of Upland Farming, Kyushu National Agricultural Experiment Station, MAFF, 6644 Yokoichi, Miyakonojo, Miyazaki 885 , Japan*

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Abstract

Starch samples from 51 sweet potatoes and 27 buckwheats with large variations in onset (T_o), peak (T_p) and heat (ΔH) for gelatinization determined by differential scanning calorimetry (DSC) were studied. Amylose content was found to be independent of all the DSC parameters. The quantitative molar distributions of the amylopectin chain length (DP 6–17) analyzed by high performance anion-exchange chromatography had a large influence on all the DSC parameters. It is concluded that, within the same botanical origin, an increase in short outer chains in amylopectin molecules reduces the efficiency of packing within the crystalline region of starch granules, resulting in lower T_o, T_p and ΔH.

1. Introduction

Differences in gelatinization properties are found between different plant species. Furthermore, starches from the same botanical origin can exhibit wide ranges of gelatinization properties. Large differences in gelatinization temperature have been reported among waxy rices (Tester and Morrison, 1990), and our previous study on 10 sweet potato starches (Noda et al., 1997) demonstrated that the ranges of onset (T_o), peak (T_p) and heat (ΔH) for gelatinization determined by DSC were 55.8–73.1°C, 61.3–76.0°C and 13.7–16.3 J g^{-1}, respectively. We tried to elucidate why the extensive variations in starch gelatinization properties within the same botanical origin occur by studying the molecular structure of the starches.

The distribution of the amylopectin chain length is thought to be the primary factor that influences the starch gelatinization properties for the following reason. It has been speculated that the free end of the short chain of amylopectin gives rise to a single cluster, consisting of double helices (Manners, 1989). These double helices of amylopectin molecules form the molecular order and the crystallinity in the starch granule. Starches with higher gelatinization temperatures and gelatinization heats might be expected to display a stronger crystalline structure or more molecular order (Cooke and Gidley, 1992). The alteration in the crystalline regions in starch granules, which indicates a change in the distribution of the amylopectin chain length, appeared to influence the starch gelatinization properties.

To estimate the distribution of the amylopectin chain lengths, high performance anion-exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) has been widely used in recent years (Koizumi et al., 1991; Hanashiro et al., 1996). By this technique, linear maltosaccharides released after debranching amylopectin could be separated into individual fragments. The values of the relative PAD responses of individual linear maltosaccharides (DP 6–17) were previously reported (Koizumi et al., 1991). Hence, an HPAEC study makes it possible to determine the molar distribution of the unit-chains (DP 6–17) of amylopectin. Molar distributions of the unit-chains (DP 6–17) of amylopectin display wide differences between botanical families (Koizumi et al., 1991; Hanashiro et al., 1996). Within the same botanical origin, relatively small variations in the amylopectin chain length were observed (Noda et al., 1995). However, the relationship between the starch gelatinization properties and molar distribution of the amylopectin chain length are not yet understood in detail.

In this study, 51 sweet potato and 27 buckwheat starches

* Corresponding author. Tel.: 096 (242) 1150; fax: 096 (249) 1002;E-mail: noda@knaes.affrc.go.jp

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with large differences in gelatinization properties measured by DSC were used. In addition, the amylose content and molar distribution of the amylopectin chain length (DP 6–17) by HPAEC were evaluated to determine whether the sources of such variations in the DSC properties within the same botanical origin are due to the alteration in molecular structure of the starch granules.

2. Materials and methods

2.1. Materials

Fifty-one samples of sweet potato roots differing in variety and/or cultivation conditions were used. The sweet potato samples were grown in a field at Miyakonojo, Miyazaki, Japan. Part of the sweet potato samples included those previously studied (Noda et al., 1996; Noda et al., 1997). Starch was extracted from each sweet potato root, as previously described (Noda et al., 1992). Twenty-seven kinds of buckwheat seeds (17 kinds of common buckwheat and 10 kinds of tartary buckwheat) differing in variety and/or cultivation conditions were used. The buckwheat samples were grown in a field at Nishigoshi, Kumamoto, Japan. The buckwheat seeds were ground into flour. Starch was extracted from each buckwheat sample as follows. The buckwheat flours were suspended in 0.2% sodium hydroxide at 4°C for 2 h to remove the protein and then centrifuged. The supernatant was discarded, and alkaline extraction repeated. The residue was successively washed with distilled water and passed through a 62 μm sieve. The filtrate, a starch suspension, was allowed to stand. Starch granules were decanted from the extract. Each buckwheat starch was successively washed with distilled water twice, with ethanol and acetone, and air-dried. Crystalline *Pseudomonas* isoamylase was purchased from Seikagaku Kogyo Co.

2.2. Starch properties

Differential scanning calorimetry measurements were carried out using a Perkin–Elmer DSC-7 analyzer (Perkin–Elmer Co., CT), as previously reported (Noda et al., 1996). All DSC analyses were conducted in triplicate. The blue values at 680 nm were determined according to the modified method previously reported (Noda et al., 1992), eliminating the step of starch defatting. The amylose content was calculated from the BV as reported by Takeda et al. (1983) using the BV of amylose and amylopectin described by Fujimoto et al. (1981). All determinations of amylose content were conducted in triplicate. The preparation of the isoamyloyzates of starch was carried out using crystalline *Pseudomonas* isoamylase as described earlier (Noda et al., 1995). For the fractionation of linear maltosaccharides, the HPAEC was conducted using a Dionex BioLC system (Dionex Co., CA) equipped with PAD and a CarboPac PA1 column (4 × 250 mm), as Koizumi et al. (1991) reported. Using the values of the relative PAD responses of individual linear maltosaccharides (DP 6–17), the molar distributions of the unit-chains (DP 6–17) of the amylopectins were calculated. All the HPAEC analyses were conducted in duplicate. To investigate the relationships between the molar proportions of chains and gelatinization properties of starch granules, correlations were determined between the DSC parameters and the amylase content, and the DSC parameters and the content of the amylopectin unit-chains of length from DP 6 to DP 17 were determined within the same botanical origin.

3. Results

The mean and range of the DSC parameters and amylose content in the starch samples for the 51 kinds of sweet potato roots and 27 kinds of buckwheat seeds examined in this study are given in Table 1. The sweet potato starches tended to have higher T_o, T_p and ΔH values than did the buckwheat starches; T_o, T_p and ΔH ranged from 55.7 to 73.1°C, from 61.3 to 77.6°C and from 12.7 to 16.8 J g^{-1} with mean values of 66.9°C, 72.4°C and 14.7 J g^{-1}, respectively, for sweet potato, and ranged from 51.5 to 62.3°C, from 57.2 to 66.7°C and from 9.4 to 13.9 J g^{-1} with mean values of 57.3°C, 63.5°C and 12.8 J g^{-1}, respectively, for buckwheat. Thus, these large differences

Table 1
Parameters and amylose content in starch samples of 51 sweet potatoes and 27 buckwheats

	Sample mean ± standard deviation	Range
	Sweet potato	
T_o (°C)	66.9 ± 4.5	55.7–73.1
T_p (°C)	72.4 ± 3.3	61.3–77.6
ΔH (J g^{-1})	14.7 ± 0.9	12.7–16.8
Amylose (%)	19.7 ± 2.6	14.2–24.3
	Buckwheat	
T_o (°C)	57.4 ± 2.6	51.5–62.3
T_p (°C)	63.5 ± 2.1	57.2–66.7
ΔH (J g^{-1})	12.9 ± 1.0	9.4–13.9
Amylose (%)	24.0 ± 1.5	21.1–27.4

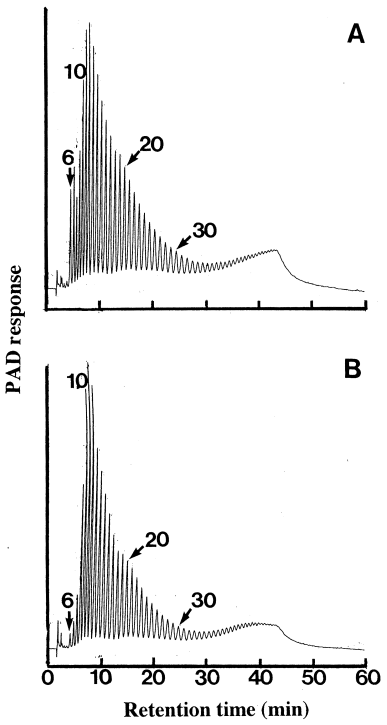

Fig. 1. HPAEC elution profiles of isoamylolyzates form typical sweet potato (A) and buckwheat (B) starches. The number on each peak represents the DP.

in the DSC characteristics in both cases of sweet potato and buckwheat were considered sufficient to allow a valid statistical analysis.

Amylose contents ranged from 14.2 to 24.3% and from 21.1 to 27.4% with mean values of 19.7% and 24.0% for sweet potato and buckwheat, respectively. The starches tested in this study did not display high (more than 30%) or low (less than 10%) amylose contents.

HPAEC chromatograms of isoamylolyzates from typical sweet potato and buckwheat starches are presented in Fig. 1. The number on each peak represents the DP. The unit-chains of debranched starches were distributed between DP 6 and >40. The chromatograms produced very different profiles among the two different plant species. Fig. 2 shows the average values of molar distributions of the unit chain (DP 6–17) of amylopectins for sweet potato and buckwheat. Each sweet potato amylopectin showed a distribution with a broad peak at DP 11–13 and a trough at DP 8. Each buckwheat amylopectin had a peak at DP 11. The amounts at DP 6 and 7 were low for buckwheat amylopectin, while the proportions of DP 6 and 7 were relatively high for sweet potato amylopectin. These data indicate that the molar distributions of the unit chains (DP 6–17) differ between the sweet potato and buckwheat amylopectins. On the other hand, the differences in the amylopectin chain length were small within the same botanical origin. The most striking points were the wide variations in the amounts of DP 6–8 for sweet potato and the wide variations in the amount of DP 9 for buckwheat. The trends obtained for sweet potato were in good agreement with our previous study (Noda et al., 1995). Correlations between amylose content, each content of the unit-chains (DP 6–17) of amylopectin and DSC parameters were calculated to determine how the variations that existed in these parameters influenced one another (Table 2). For sweet potato and buckwheat, no significant correlations were detected between the amylose content and all the DSC parameters, T_o, T_p and ΔH, indicating that the amylose content was independent of the starch gelatinization properties determined by DSC.

The molar distributions of amylopectin determined by HPAEC were found to have profound effects on all the DSC parameters for sweet potato and buckwheat. Although the contents of DP 6 were negatively correlated with all the DSC parameters at the 1% level for sweet potato, no significant correlations existed between the contents of DP 6 and all the DSC parameters for buckwheat. The negative correlations of the contents of DP 7 with all the DSC parameters were at the 1% level for sweet potato and buckwheat. However, the fact that the contents of DP 7 and variations in the contents of DP 7 were small for buckwheat suggested that the correlations obtained for buckwheat were not very

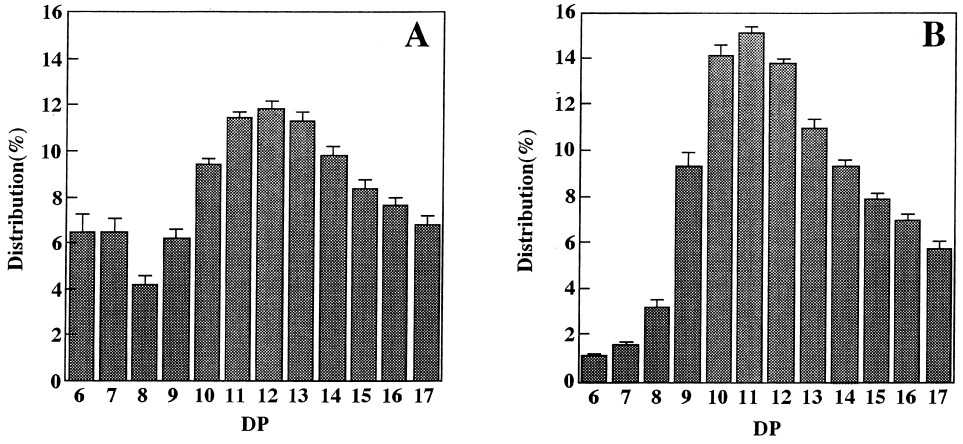

Fig. 2. Average values of molar distributions (% of total) of amylopectin unit-chains with DP 6–7 for sweet potato (A) and buckwheat (B). Error bars indicate standard deviation.

Table 2
Correlations between DSC parameters and amylose content or each content of unit-chain with DP 6–17 of amylopectin for sweet potato and buckwheat

	Sweet potato			Buckwheat		
	T_o	T_p	ΔH	T_o	T_p	ΔH
Amylose	−0.094	0.032	0.113	−0.056	−0.010	0.147
DP6	−0.745**	−0.724**	−0.496**	−0.002	0.166	0.253
DP7	−0.720**	−0.749**	−0.637**	−0.812**	−0.886**	−0.526**
DP8	−0.337*	−0.471**	−0.363**	−0.551**	−0.694**	−0.563**
DP9	−0.198	−0.346*	−0.367**	−0.619**	−0.733**	−0.496**
DP10	−0.332*	−0.424**	−0.570**	−0.659**	−0.710**	−0.301
DP11	0.039	0.075	0.153	−0.627**	−0.561**	−0.063
DP12	0.450**	0.554**	0.346*	0.072**	0.229	0.325
DP13	0.559**	0.671**	0.516**	0.604**	0.700**	0.415*
DP14	0.599**	0.685**	0.655**	0.783**	0.823**	0.403*
DP15	0.617**	0.659**	0.659**	0.839**	0.850**	0.408*
DP16	0.644**	0.662**	0.630**	0.760**	0.806**	0.402*
DP17	0.705**	0.698**	0.628**	0.679**	0.744**	0.381*

**Significant at 0.01 level
*Significant at 0.05 level

meaningful. For sweet potato, relatively small effects of the amounts of DP 8 and 9 on all the DSC parameters, for example, showed no significant correlation between the amounts of DP 9 and T_o. On the contrary, the negative correlations between the amounts of DP 8 and 9 with all of DSC parameters were at the 1% level for buckwheat. There were no significant correlatations between the contents of the intermediate chain length, which was DP 11 for sweet potato and DP 12 for buckwheat, and all the DSC parameters. For sweet potato, each proportion of the unit-chains in the range of DP 12–17 was positively correlated with all the DSC parameters at the 1% level except for the correlation of the proportion of DP 12 with ΔH, which was at the 5% level. Similarly, the positive correlations of each proportion of the unit-chains in the range of DP 13–17 with T_o and T_p were at the 1% level and those with ΔH were at the 5% level. It was found from our results that, with some exceptions, the amounts of the shorter unit-chains, which were DP 6–10 for sweet potato and DP 6–11 for buckwheat for amylopectin, were negatively correlated with all the DSC parameters, T_o, T_p and ΔH, while those of longer unit-chains, which were DP 12–17 for sweet potato and DP 13–17 for buckwheat, were positively correlated.

4. Discussion

Fifty-one samples of sweet potato starches and 27 samples of buckwheat starches examined in this study exhibited very different temperatures and heats for gelatinization. This study was intended to determine how such variations occurred within the same botanical origin in terms of macromolecular structure of starch granules, not of genetic and/or environmental factors. The statistical analysis presented here represents the first assessment of interactions between macromolecular architecture and gelatinization properties using a large number of starch samples extracted from two botanical species.

Amylose content (amylose-to-amylopectin ratio) and distribution of the amylopectin chain length were included in this study for the molecular characteristics of the starch granules. Gel-permeation chromatography (Ikawa et al., 1978) and high performance size exclusion chromaorgarphy (Hizukuri, 1986) have been frequently used for determining the distribution of the amylopectin chain length. However, maltosaccharides released after debranching cannot be divided into the individual members using these techniques. HPAEC equipped with PAD is satisfactory for separation of these maltosaccharides into individual members, and the values of the relative PAD responses have been documented up to DP 17 (Koizumi et al., 1991). Here, we used HPAEC to obtain knowledge of the distribution of the amylopectin chain length (DP 6–17) on a molar basis.

In this study, we used intact starch, a mixture of amylose and amylopectin, for debranching with isoamylase. Amylose was reported to have a small amount of branched molecules (Takeda et al., 1992). The HPAEC patterns of isoamylolyzates of wheat starch and its purified amylopectin have been shown to be similar (Nagamine and Komae, 1996), clearly showing that the levels of small chains from amylose are too small to have an influence on the distribution analysis of the amylopectin chain length, and that it is possible to use intact starch instead of purified amylopectin.

This is the first report of examination of the chain length distribution of buckwheat amylopectin, resembling that for wheat amylopectin, exhibiting a small amount of DP 6–8 and a somewhat sharp peak at DP 11 (Koizumi et al., 1991; Hanashiro et al., 1996). The distributions of amylopectin chains analyzed by HPAEC were reported to differ largely according to its botanical origin (Koizumi et al., 1991; Hanashiro et al., 1996), which agrees with our present

results on the amylopectins from sweet potato and buckwheat. The distributions of the amylopectin chains appear to have taxonomic significance as genetic markers for their diversity.

According to the cluster model of amylopectin proposed by Hizukuri (1986), amylopectin chains can be fractionated into B_3, B_2, B_1 and A chains. A chains carry no chain, and B chains carry at least one or more A or B chains. A and B_1 chains are localized to one cluster, B_2 chains span two clusters and B_3 chains span three clusters. A single cluster displays the double helical structure, forming the crystallinity of starch granules. The length of the crystalline region is 6 nm, which corresponds to the length of 18 glucose residue (Ball et al., 1996). Unit-chains with DP 6–17, which would mainly comprise A chains, would give rise to a single cluster of amylopectin. There is only limited information concerning the contribution of the amount of the short unit-chains with the DP 6–17 of amylopectin to starch gelatinization properties. For this reason, we tried to study the relationships between each amount of the individual unit-chains with the DP 6–17 of amylopectin and all the DSC parameters. Results of the statistical analysis support the idea that the molecular structure of amyloectin influences starch gelatinization. Clearly, the most important point is that, in general, the amounts of shorter chains of amylopectin were negatively correlated with all the DSC parameters, T_o, T_p and ΔH for sweet potato and buckwheat. It is well known that the stability of an individual double helix, which means gelatinization temperature and gelatinization heat, will increase with increasing helix length. This is considered to be the reason for the observed correlation. According to the report of Shi and Seib (1995), who studied the physicochemical properties of maize starches from the *wx*, *du wx*, *ae wx* and *ae du wx* genotypes, *ae wx* starch had the lowest proportion of short chains with DP 6–11, in addition to the highest gelatinization temperature and gelatinization heat. These results also support the assumption mentioned above. Higher proportions of the short chains with the DP 6–9 of amylopectin were found to inhibit retrogradation (Wursch and Gumy, 1994). The second important point is that differences in the DP of unit-chains have an impact on DSC-parameters among sweet potato and buckwheat amylopectins. Specifically, among the short unit-chains with DP 6–11, the contribution of DP 6 and 7 to the DSC parameters were large for sweet potato, while the contents of DP 8 and 9 were large for buckwheat.

As the crystalline region of the starch granules are generally composed of amylopectin and not of amylose, starches with the higher amylose content are assumed to show lower T_o, T_p and ΔH because of the lower proportion of the crystalline region. However, it is clear from our results that the amylose-to-amylopectin ratio did not correlate with any of the DSC parameters. Similar results were observed in our previous report (Noda et al., 1993). For 34 rice starches, no significant correlation existed between amylose content and gelatinization temperature (Juliano and Perez, 1990). Thus, it is suggested that DSC parameters, T_o, T_p and ΔH, is influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains, not by the proportion of crystalline region, which corresponds to the amylose-to-amylopectin ratio.

Our results explain some of the variations in starch gelatinization properties that occur within the same botanical origin. It follows that starch with a lower T_o, T_p and ΔH had relatively abundant shorter unit-chains of amylopectin within the same botanical origin. Consequently, starch gelatinization reflects the profile of the molecular architecture of amylopectin but not the amylose-to-amylopectin ratio. Other sources of differences in starch gelatinization properties between different botanical origin remain to be elucidated

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