Quality Characteristics of Waxy Hexaploid Wheat *(Triticum aestivum* L.): Properties of Starch Gelatinization and Retrogradation

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ABSTRACT

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The viscoelastic properties and molecular structure of the starch isolated from waxy (amylose-free) hexaploid wheat (WHW) *(Triticum aestivum* L.) were examined. WHW starch generally had lower gelatinization onset temperature, peak viscosity, and setback than the starch isolated from normal hexaploid wheat (NHW). Differential scanning calorimetry (DSC) showed that WHW starch had higher transition temperatures (T_o, T_p, and T_c) and enthalpy (ΔH) than NHW starch. However, when compared on the basis of amylopectin (AP) content, ΔH of WHW starch was almost statistically identical to that of its parental varieties. Typical A-type X-ray diffraction patterns were observed for the starches of WHW and its parental varieties. Somewhat higher crystallinity was indicated for WHW starch. WHW starch was also characterized by having greater retrogradation resistance. The high-performance size-exclusion chromatography (HPSEC) of amylopectin showed that each amylopectin yielded two fractions after debranching. Although WHW amylopectin had somewhat long B chains, little difference was observed in the ratio of Fr.III/Fr.II between WHW and its parental varieties.

Recently, many gene-encoding enzymes that take part in starch synthesis have been identified. These efforts have led to the generation of new phenotypes which are characterized by having different starch synthesis characteristics. The properties of these starches have been reported (Stark et al 1992, Visser and Jacobsen 1993, Müller-Röber and Koßmann 1994, Shewmakar et al 1994). The Wx protein, assumed to be the granule-bound starch synthase (GBSS, EC 24.1.21), is a key enzyme in the synthesis of amylose (Preiss 1991). Waxy mutants lacking this enzyme have been identified in several cereals, including maize, rice, barley, sorghum, and amaranth. The structure and property of these waxy starches are well documented. By using differential scanning calorimetry (DSC) and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), Shi and Seib (1992) assessed the relationship between the fine structure of various types of waxy starches and their gelatinization and retrogradation properties. Salomonsson and Sundberg (1994) estimated the profile of amylopectin (AP) from waxy barley starch by gel-permeation chromatography (GPC). They quantitatively separated starch by molecular weight distribution and showed mean degree of polymerization of AP. Though a great deal of effort was spent on breeding waxy hexaploid wheat (WHW), no one has yet succeeded in developing a commercial variety. Nakamura et al (1992) suggested that wheat cultivars lacking in high molecular weight (HMW) Wx protein would have potential as an important source for the breeding of WHW cultivars. They succeeded in detecting two types of wheat cultivars (Kanto 107 [Kl07], and Bai-Huo) which partially lacked the Wx protein. By crossing K107 and Bai-Huo, they finally succeeded in the breeding of WHW (Nakamura et al 1995). This investigation, therefore, has focused on the functional and structural properties of the WHW starch.

MATERIALS AND METHODS

Wheat

Waxy hexaploid wheat *(Triticum aestivum* L.) (F4 seeds), lacking all of three Wx proteins (Wx-Al, Wx-Bl, and Wx-Dl) and showing waxy phenotype, were grown at Tohoku National Agricutural Experimental Station (Morioka, Japan) in 1995. The parental varieties (Kl07 and Bai-Huo) and two registered Japanese wheat cultivars (Norin 61 [N61] and Norin 126 [Chihokukomugi: N126]) were grown at the station in 1995. WHW was grown in a greenhouse, and the others were grown in an experimental field. Both N61 and N126 are recognized to have the best noodle-making quality of all registered Japanese wheat cultivars.

Starch Samples

Starch was prepared according to the methods described by Endo et al (1991). Wheat samples were ground to pass through an 80-mesh sieve in a coffee grinder. Dough balls were prepared from 30 g of ground wheat samples and 21 mL of water. After resting the dough balls in cold water for 30 min, starch was washed from them with water. The starch suspension was passed through a 100-mesh nylon sieve to remove particles of bran and gluten. Starch milk suspensions were then centrifuged (3,000 × g, 20 min). Isolated starch samples were freeze-dried. To minimize the effect of lipids on functional properties of starch, the dried samples were defatted at room temperature; they were dispersed and stirred in methanol for 2 hr, and then the process was repeated using ethanol.

Normal maize *(Zea mays* L.) and waxy maize starch used in this study were available commercially (Nihon Shokuhin Kakou, Tokyo). These were defatted using the same procedure described above. Amylose contents of the isolated starch samples were determined by a modified method as described by Williams et al (1970).

Viscosity

Starch viscosity was analyzed on a Brabender Visco/Amylograph (type VA-1B) and on a Rapid Visco Analyser (RVA model 3D, Newport Scientific, Narrabeen, Australia). Amylograms were run in the viscoamylograph with a 700-g·cm sensitivity cartridge using 44.7 g of starch (dry basis) and 450 mL of water. The temperature was raised from 25 to 95°C, held at 95°C for 10 min, and then cooled to 63°C at a rate of 1.5°C/min. Pasting viscosity of 4 g of starch in 25 mL of water was analyzed using the RVA (held 2 min

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at 60°C, heated to 95°C at 6°C/min, held 4 min at 95°C, cooled to 50°C at 11.3°C/min, and finally held 4 min at 50°C). In addition to peak viscosity, breakdown and setback were calculated.

Preparation of Amylopectin

Amylopectin was prepared according to the methods described by Banks and Greenwood (1967). We used butanol and *n*-hexanol as precipitants of amylose. The purity of each amylopectin was tested by using iodine affinity value (Schoch 1964).

High-Performance Size-Exclusion Chromatography

Digests containing amylopectin (20 mg) and isoamylase (EC 3.2.1.68) from *Pseudomonas amyloderamosa* (600 U, Sigma Chem. Co., St. Louis, MO) in acetate buffer (5 mL, 10 m*M*, pH 3.8) were incubated at 40°C for 24 hr and then boiled for 10 min to inactivate the enzyme. Before high-performance size-exclusion chromatography (HPSEC) analyses, the debranched amylopectin solutions were stored at 40°C to prevent the formation of insoluble materials. The chain distribution of debranched amylopectins was estimated by using an HPSEC system that included a HPLC-pump (L-6200, Hitachi, Japan), autosampler (655A-40, Hitachi), degasser (ERC-3310, Erma Optical Works, Japan), and a differential refractometer (Shodex RI se-61, Showa-Denko, Japan). The system also included a guard column of 6.0 mm i.d. × 40 mm and two columns of 7.5 mm i.d. × 300 mm (Showa-Denko). Columns and detector were maintained at 60°C. Distilled water used as eluent was filtered with a Millipore filter (0.45 μm) and degassed (0.5 mL/ min flow rate). An aliquot (20 μL) of digested amylopectin solution was injected into the HPSEC system. For calibration of the column system, a solution of the TSK standard, Poly (ethylene oxide) (Showa-Denko) of different molecular weights ($2.1–16 \times 10^4$) was used.

Average Degree of Polymerization of Amylopectin

The solutions of amylopectin were assayed for total carbohydrate (phenol-sulfuric acid method) and nonreducing sugar by using the rapid Smith-degradation method, as described by Hizukuri and Osaki (1978). Both methods were calibrated against glycerol. Average degree of polymerization (DP) was determined by dividing the total amount of polysaccharide by the nonreducing residue.

Physical Measurements

Differential scanning calorimetry (DSC) studies were performed with a 560U system (Daini-Seikosha, Japan). Native starch slurry samples (1:4 starch-to-water ratio), sealed in silver sample cells, were heated from 25 to 150°C at 5°C/min. Enthalpy (ΔH) was determined by measuring the area of the DSC endotherm. The temperature of the characteristic transitions at onset (T_o), peak (T_p), and completion (T_c) were recorded. Measurements on the amylose-lipid endotherm in the 94–120°C range also were made.

Structural changes during starch retrogradation were analyzed according to the method described by Nakazawa et al (1985). After the first scan, the sample cells were stored for one, two, and three weeks at 4°C. Stored samples were then examined by DSC.

Native, gelatinized (prepared by suspending the native starch in 10-fold volume of distilled water and boiling for 30 min), and retrograded (gelatinized samples stored for one, two, and three weeks at 4°C) starch samples (500 mg) were investigated on an X-ray diffractometer (D/MAX-A, Rigaku-Denki, Japan). After samples were moistened by incubating for 16 hr at 90% rh, they were scanned from 5 to 30° at a rate of 4.8°/min.

RESULTS AND DISCUSSION

Viscoelastic Properties

Amylose content was analyzed by iodine affinity. Because the reagent grade of potato amylopectin (Sigma) showed a faint absorbance, data were corrected on the basis of background absorbance. As a result, apparent amylose contents obtained in this study were

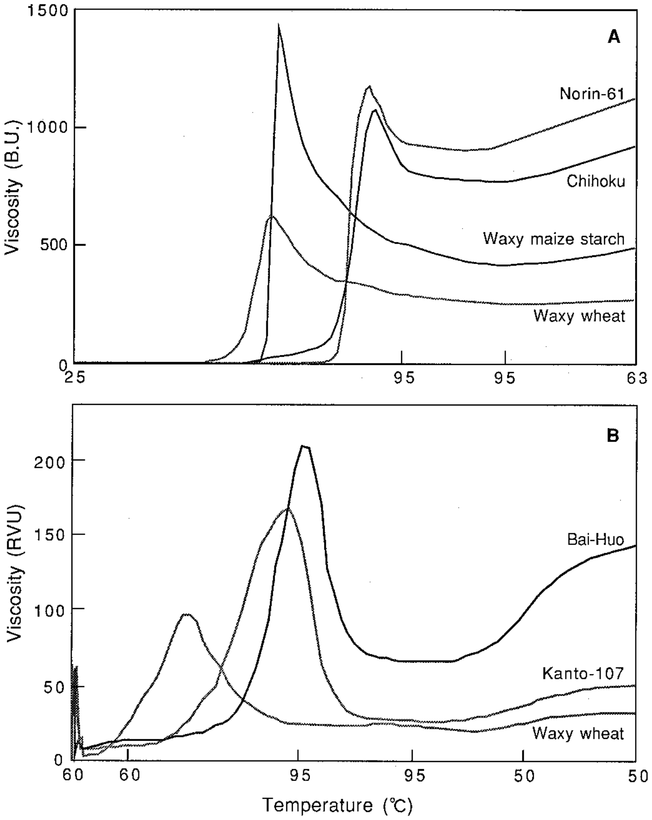

Fig. 1. Comparison of viscoelastic properties for the starch isolated from normal hexaploid wheat varieties, waxy hexaploid wheat, parental varieties (Kanto-107 and Bai-Huo), and waxy maize starch. **A,** Brabender Visco/ Amylograph. **B,** Rapid Visco Analyser.

TABLE I
Amylose Contents (%) of Starch and Properties of Fractionated Amylopectin

				Fractionated Amylopectin	
Source	Type[a]	Variety	Native Starch	FrIII/FrII[b]	DP[c]
Wheat	N	Norin-61 (N61)	23.0 ± 1.1	1.17 ± 0.08	21 ± 1
	N	Chihoku (Nl26)	24.3 ± 1.6	1.17 ± 0.04	25 ± 2
	N	KantolO7 (KlO7)	16.6 ± 1.0	0.97 ± 0.14	22 ± 1
	N	Bai-Huo	24.2 ± 0.9	1.06 ± 0.08	22 ± 1
	W	Waxy wheat	0.0	1.15 ± 0.07	25 ± 1
Maize	N	Normal maize	26.3 ± 0.3	0.83 ± 0.08	28 ± 2
	W	Waxy maize	0.0	0.59 ± 0.10	23 ± 1

[a] N = normal starch, W = waxy starch.

[b] Peak area ratio obtained from the analysis of high-performance size-exclusion chromatography for the amylopectin fractions debranched with isoamylase.

[c] Degree of polymerization, average chain length for the amylopectin fractions debranched with isoamylase.

somewhat lower than those of previous studies (Oda et al 1992, Yamamori et al 1992) (Table I). Nakamura et al (1995) showed that WHW lacks Wx-Al, -Bl and -Dl proteins, but N61 has all these three Wx proteins. N126, K107, and Bai-Huo also lacked Wx-Bl, -Al, and -Bl, and -Dl, respectively.

Amylogram tests revealed that WHW starch possessed the typical viscoelastic property of waxy sources. As shown in Fig. 1A, waxy starch (WHW starch and waxy maize starch) swelled rapidly and took less time to reach peak viscosity than normal starches. Waxy starch also showed less setback than normal starch (NHW and normal maize starch). Some difference was observed between WHW starch and waxy maize starch, with the exception of peak viscosity value where WHW starch showed the lowest value. Because the difference in viscoelastic property was not considered simply the result of amylase activity (*data not shown*), WHW starch was further compared with its parental varieties (Kl07 and Bai-Huo). An RVA was used to confirm the effects of pedigree lines on the property of WHW starch. As shown in Fig. lB however, little relation of the pedigree lines to viscosity was found. Consequently, the viscoelastic property observed for the WHW starch appeared not to be inherited.

Starch gelatinization was proposed to take place in two stages (Schoch 1965). The first stage is the swelling of the starch granules by imbibing water (Schoch 1965, Collison 1968). Both the swelling and the solubilization of starch molecules are included in the second stage. An amorphous region of starch granules swells faster than a crystalline region. The swelling power is enhanced when a crystalline region begins to melt. Tester and Morrison (1990) also indicated that the amylopectin fraction was responsible for the swelling power. If the system is heated above its gelatinization temperature, the starch granules are ruptured, and a gel or a paste is formed during cooling, with interaction among amylose and amylopectin. Accordingly, it was considered that rapid swelling resulted from the low amylose content (amylose-free). The low setback value of WHW starch may also have been because the starch lacked amylose that recrystallized irreversibly during cooling. Because amylose is required for forming a continuous gel matrix (Ring and Stainby 1982, Ring 1985), waxy samples were unable to develop this structure. The low peak viscosity in the WHW starch was thought to be caused by a weak gel matrix, but the reason remains obscure.

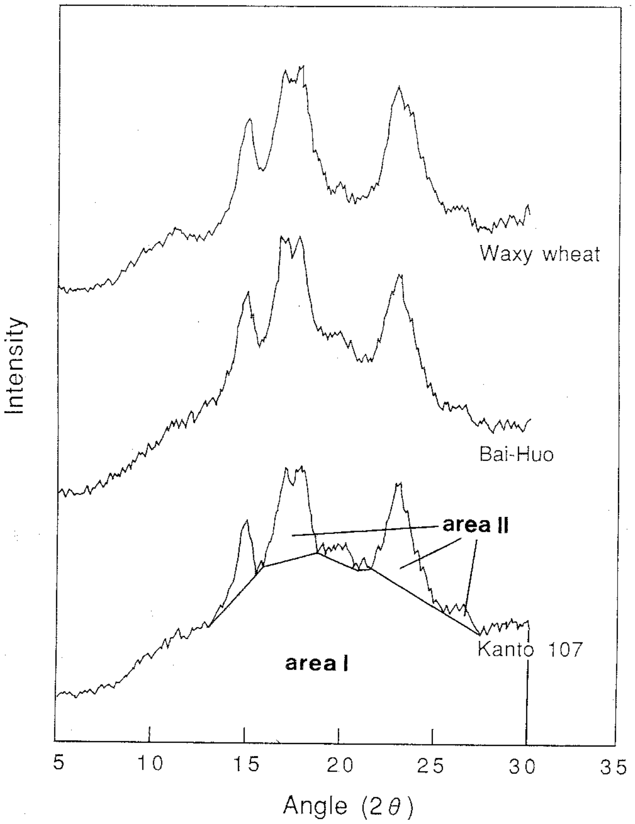

Fig. 2. Comparison of X-ray diffractograms for starch isolated from waxy hexaploid wheat and parental varieties (Kanto-107 and Bai-Huo).

Physical Properties

The typical A-type patterns of X-ray diffractograms were observed for all starches (Fig. 2). WHW starch showed somewhat lower intensity peak at $2\theta = 20.2°$. Because results of X-ray diffractograms are closely related to starch granule crystallinity, a crystalline or amorphous region of starch granules could be estimated by comparing the areas under principal peaks. The area between a flat horizontal baseline and a tail-to-tail baseline of each peak must represent the amorphous region (area I). On the other hand, the crystalline region must be estimated by the sum of the surrounding total area between each peak and a tail-to-tail baseline (area II). A somewhat lower value for the ratio of area I to II was observed for WHW starch than for those of its parental varieties (*data not shown*). These results suggested that there might be some structural differences between WHW starch and its parental varieties by the lack of amylose component.

Figure 3 shows a DSC thermogram for K107 starch. Transition temperatures (T_o, T_p, and T_c) and enthalpies (ΔH) are summarized in Table II. Neither WHW nor waxy maize showed a peak at 100°C. These results might be related to the lack of amylose component. According to Eliasson (1994), a larger peak at 64°C and a smaller peak at 100°C were related to the gelatinization of amylopectin and the dissociation of the lipid-amylose complex, respectively. Waxy starch exhibited higher ΔH values than did

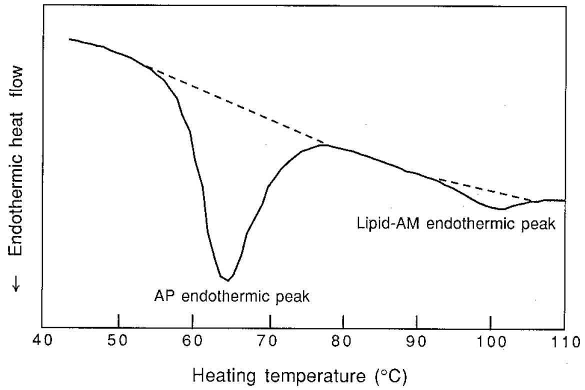

Fig. 3. Typical differential scanning calorimetry thermogram for the starch isolated from Kanto-107. AM: amylosse, AP: amylopectin.

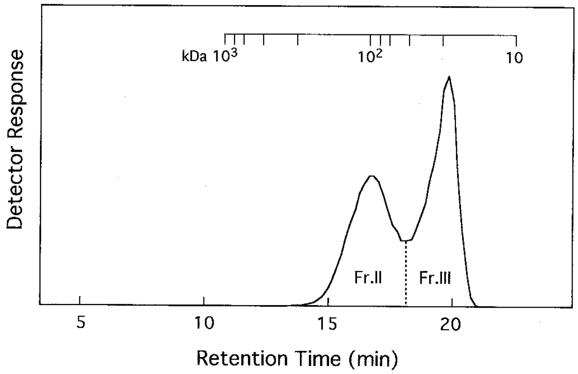

Fig. 4. Typical high-performance size-exclusion chromatogram for the isolated amylopectin fractions (Kanto-107) after debranching with isoamylase.

normal starch. The results of DSC analysis support the X-ray diffraction data of WHW starch showing more crystallinity than NHW. However, it should be noted that little difference was observed when the data were evaluated on the basis of amylopectin content (Δ*H*/AP). Similar results were indicated for barley (Morrison et al 1993), rice (Biliaderis et al 1986), and maize (Inouchi et al 1991a). Furthermore, differences in transition temperatures between normal and waxy starches varied among botanical sources for wheat or maize. Within wheat varieties, WHW starch showed the highest T_o, T_p, and T_c. There were few differences between waxy and normal maize starch. Again, little relation of the pedigree lines to DSC parameters of WHW starch was observed (compare WHW, K107, and Bai-Huo).

HPSEC

Kobayashi et al (1986) showed that three peaks originating from amylopectin existed in the HPSEC chromatogram after debranching wheat starch with isoamylase, but only two peaks (longer chain fraction II and shorter chain fraction III) were detected (Fig. 4) from the debranched amylopectin with isoamylase in this study. Because the major peak observed before debranching disappeared after debranching, the amylopectins of all specimens were considered to be completely digested (data not shown). No amylose (fraction I) existed. According to the amylopectin model proposed by Hizukuri (1986), B_2 and B_3 chains were included in fraction II, and A and B_1 chains were included in fraction III. Table I shows the peak area ratio of fraction III to fraction II (Fr. III to Fr. II) and the average chain length (DP) of fractionated amylopectins.

All DP values obtained in this study were within the ranges reported by Biliaderis et al (1981) and Hizukuri (1985). Waxy maize starch had a lower ratio of Fr.III to Fr.II and lower DP than did normal maize starch, suggesting that waxy maize starch had less branching and more short chains than normal maize starch. In contrast, within wheat varieties there were few differences in the values of Fr.III to Fr.II. The ratio in WHW starch was greater than the ratio of the parental varieties (Kl07 and Bai-Huo) but was generally similar to N61 and N126. WHW had significantly higher DP values than its parental varieties ($P < 0.025$). These results suggested the amylopectin structure in WHW was not characterized by the features of amylopectin in its parental varieties but by the lack of waxy protein. To confirm this observation, more studies, including DP analysis, would be needed.

Retrogradation Properties

The change in starch during retrogradation was analyzed by X-ray diffractometry and DSC. As shown in Fig. 5, all gelatinized starches showed Verkleisterungs spectrum. No remarkable peak in X-ray diffractogram could be distinguished in any of the freshly gelatinized starches, indicating that gelatinization was complete under the experimental conditions used. An increase in crystallinity, however, was observed when the gelatinized starch was stored for a long time at 4°C. The longer the storage time of starch at 4°C, the larger the peak at 2 $\theta = 17°$ (4a rings). Compared to normal wheat starch, WHW starch showed little increase in 4a rings. Similar results were obtained for waxy rice starch (Takeda and Hizukuri 1974).

Because the starch samples were completely gelatinized, thermal transitions were not observed for DSC runs of freshly gelatinized starches (data not shown). When gelatinized starch samples were stored at 4°C, an endothermic transition was again observed (data not shown). These results were attributed to the retrogradation of amylopectin. The transition temperature was lowered, and the shape of the endothermic peak widened, which suggests that the structure of retrograded starch is different from native starch, as has been indicated by Inouchi et al (1991b). Figure 6 shows remarkable increases in Δ*H* values when starch samples were stored for up to three weeks at 4°C. Waxy starch showed less increase than normal starch. These results were in accordance with the results obtained from X-ray diffractometry and indicated the resistance of WHW starch to retrogradation. Further studies, including storage conditions and water addition levels, would be needed to confirm the retrogradation resistance of WHW starch. Gudmundsson (1994) reviewed retarding or interfering factors to retrogradation such as storage temperature, water content, and presence of lipids or surfactants.

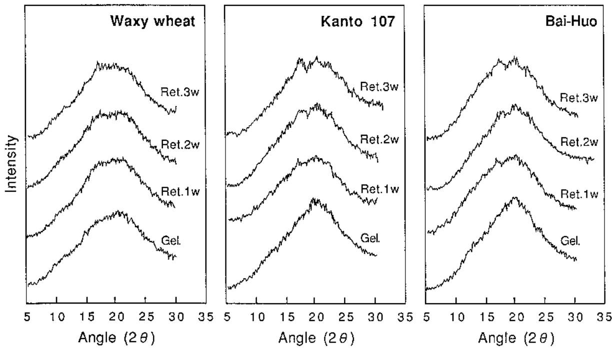

Fig. 5. Comparison of X-ray diffractograms for the freshly gelatinized starch between waxy hexaploid wheat and normal hexaploid wheat (parental varieties Kanto-107 and Bai-Huo) and their changes during storage at 4°C. Gel = freshly gelatinized starch; Ret.1w, Ret.2w, and Ret.3w = stored for one, two, and three weeks, respectively, at 4°C.

TABLE II
Thermal Transition Temperatures (°C)[a] and Enthalpies[b] of Various Starches

			Amylopectin (AP) Endotherm[d]					Amylose-Lipid Endotherm[d]	
Source	Type[c]	Variety	T_o	T_p	T_c	Δ*H*	Δ*H*/AP	T_p	Δ*H*
Wheat	N	Norin-61 (N61)	57.6	62.4	73.7	6.1	7.7	100.1	0.7
			(1.2)	(0.1)	(0.5)	(0.4)	(0.5)	(0.4)	(0.1)
	N	Chihoku (Nl26)	58.7	64.3	75.1	6.3	8.2	100.7	0.4
			(0.1)	(0.2)	(0.6)	(0.3)	(0.4)	(0.4)	(0.0)
	N	Kanto l07 (Kl07)	59.0	64.1	78.1	7.6	8.9	100.3	0.4
			(0.4)	(0.3)	(1.0)	(0.5)	(0.6)	(0.4)	(0.1)
	N	Bai-Huo	56.2	62.2	76.5	7.4	9.5	100.0	0.4
			(0.6)	(0.2)	(0.5)	(0.3)	(0.4)	(0.4)	(0.1)
	W	Waxy wheat	59.8	65.6	83.6	9.1	9.1	no peak	
			(0.4)	(0.2)	(0.7)	(0.5)	(0.5)		
Maize	N	Normal maize	68.1	72.7	85.8	7.9	10.4	101.8	0.4
			(0.1)	(0.3)	(2.3)	(0.5)	(0.6)	(0.9)	(0.1)
	W	Waxy maize	65.3	71.8	85.7	9.8	9.8	no peak	
			(0.2)	(0.2)	(1.1)	(0.6)	(0.6)		

[a] T_o = onset, T_p = peak, T_c = completion.
[b] J/g of starch and amylopectin, respectively.
[c] N = normal starch, W = waxy starch.
[d] Values are the average of six separate determinations, with standard deviation in parentheses.

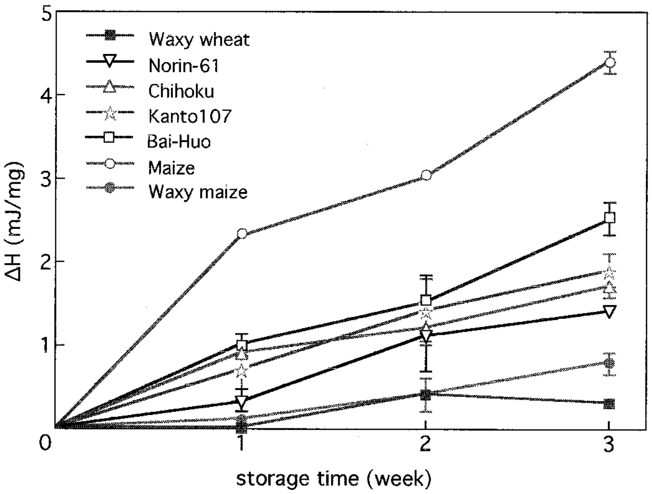

Fig. 6. Changes of enthalpy (Δ*H*) values analyzed by DSC for starches stored at 4°C after complete gelatinization. Bars denote standard deviation.

CONCLUSIONS

The viscoelastic properties of WHW starch were distinctly different from those of waxy maize starch and NHW starch. It is presumed that the difference comes from differences in both molecular structure and crystallinity between the two groups. Further studies are needed to assess the practical application of WHW and the unique properties of WHW for food production.

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