

# Comparison of Physical Properties of Wheat Starch Gels with Different Amylose Content

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## ABSTRACT

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The effects of amylose content and other starch properties on concentrated starch gel properties were evaluated using 10 wheat cultivars with different amylose content. Starches were isolated from grains of two waxy and eight nonwaxy wheat lines. The amylose content of waxy wheat lines was 1.4–1.7% and that of nonwaxy lines was 18.5–28.6%. Starch gels were prepared from a concentrated starch suspension (30 and 40%). Gelatinized starch was cooled and stored at 5°C for 1, 8, 16, 24, and 48 hr. The rheological properties of starch gels were studied by

measuring dynamic viscoelasticity with parallel plate geometry. The low-amylose starch showed a significantly lower storage shear modulus ( $G'$ ) than starches with higher amylose content during storage. Waxy starch gel had a higher frequency dependence of  $G'$  and properties clearly different from nonwaxy starches. In 40% starch gels, the starch with lower amylose showed a faster increase in  $G'$  during 48 hr of storage, and waxy starch showed an extremely steep increase in  $G'$ . The amylose content and concentration of starch suspension markedly affected starch gel properties.

Starch is the major constituent of wheat (*Triticum aestivum* L.) endosperm and an important structural component in many products made from wheat flour. Most starches in processed foods are heated in the presence of water. When heated with water, starch swells and is gelatinized. During storage, glucose polymer chains in the gelatinized starch start to reassociate in an ordered structure and form a viscoelastic gel if the starch concentration is high enough (Miles et al 1985). These changes in starch structure, known as retrogradation, decrease the eating quality of starch-based products (Biliaderis and Zawistowski 1990; Keetles et al 1996a).

Most starches consist of two polysaccharides, amylose and amylopectin. Amylose is an essentially linear molecule consisting of  $\alpha$ -(1-4)-linked D-glucan chains. Amylopectin is a highly branched molecule containing short chains of  $\alpha$ -(1-4)-linked D-glucan chains with  $\alpha$ -(1-6)-linked branches. The amylose content greatly influences the physicochemical properties of starch such as gelatinization, retrogradation, and gelation (Parovuori et al 1997; Czuchajowska et al 1998; Fredriksson et al 1998; Yuryev et al 1998). Amylose synthesis depends mainly on the waxy protein, granule-bound starch synthase (GBSS) (Tsai 1974; Echt and Schwartz 1981). Several waxy wheats lacking three waxy proteins (Wx-A1, Wx-B1, and Wx-D1) were produced by cross-breeding or mutation (Nakamura et al 1995; Yamamori et al 1995; Kiribuchi-Otobe et al 1997; Yasui et al 1997). If only one or two loci are null, wheat starch has a reduced percentage of amylose (Yamamori et al 1992, 2000; Nakamura et al 1993). Using waxy wheats and low-amylose wheats lacking one or two waxy proteins, wheat starch with varied amylose content are available. We compared the rheological properties of starch gels with different amylose content and analyzed the effect of amylose content and other starch properties on concentrated starch gel properties. Because the starch concentration is high in wheat products, measurement of concentrated starch system could provide useful information on food quality.

## MATERIALS AND METHODS

### Samples

Two waxy wheat lines (*Triticum aestivum* L.) and eight nonwaxy lines were grown in 1999 at the National Agriculture Research Center

(NARC), Tsukuba, Japan. H1881 and K107Wx2 are waxy lines with no waxy proteins. Kanto 107, Kanto 117, Kanto 119, and Kanto 122 are low-amylose lines lacking Wx-A1 and Wx-B1 proteins. Cadoux and Eradu are Wx-B1 protein-deficient cultivars. Norin 61 and Bandouwase have three waxy proteins. Cadoux and Eradu were grown sheltered from rainfall for two months before harvesting.

### Starch Isolation

All wheat grains were milled using a Quadrumat Jr. test mill with a 70GG mesh sieve after adjusting moisture content to 14% on a dry basis. Starch was isolated using the dough-ball method of Wolf (1964). Flours were fractionated into prime and tailing starches. After excluding tailing starches, prime starch was washed with 0.1M NaCl and freeze-dried. Protein content of isolated starches was 0.4–0.5 %.

### Chemical Analysis

Amylose content of isolated starch was determined by the method of Gibson et al (1997) using an amylopectin-amylose assay kit (Megazyme International Ireland Ltd., Ireland). The chain length distribution of amylopectin in starch was analyzed with high-performance anion-exchange chromatography (Dionex, CA) equipped with a pulsed-amperometric detector. The column used was a CarboPac PA-1 (250 × 4 mm i.d., Dionex) with a CarboPac PA-1 guard column (50 × 4 mm i.d., Dionex). The sample solution was prepared using the method of Nagamine and Komae (1996). After isoamylase debranching, the sample solution was eluted at 1 mL/min with a linear gradient of 100–500 mM sodium acetate in 100 mM NaOH. Unit chains of amylopectin were classified into three groups based on the degree of polymerization (DP): 6–12, 13–34, or  $\geq 35$  (Matsuki et al 1996). The peak area ratio (%) of the three groups was then calculated.

### Swelling Power and Solubilized Starch Measurement

Swelling power and solubilized starch was determined using isolated starch by the method of Sasaki et al (2000). Total sample size was constant at 5 g, and the final ratio of starch to water was constant at 0.1 to 4.9, based on starch dry weight. Isolated starch was weighed into tubes with coated screw caps to which distilled water was added. They were placed in a shaking water bath at 70°C for 10 min and transferred to a boiling water bath. After boiling for 10 min, the tubes were cooled in cold water for 5 min and centrifuged at  $39,000 \times g$  at 5°C for 30 min. The supernatant was removed to determine solubilized starch. Swelling power was determined as sediment weight (g/g). The method of Gibson et al (1997) using an amylopectin-amylose assay kit (Megazyme) was used to determine total carbohydrates and amylose content in the recovered supernatant. In this method, the amylopectin in a solubilized starch sample was precipitated by reaction with concanavalin A (Con A) and removed by centrifugation,

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and the amylose remaining in the supernatant was determined after hydrolysis using  $\alpha$ -amylase and amyloglucosidase. Glucose was used as a standard, and the percent soluble starch and amylose were corrected for the original dry weight of the starch.

### Dynamic Viscoelasticity Measurement

The dynamic viscoelasticity of starch gel was measured using a rheometer (RheoStress RS75, Haake, Germany) with parallel plate (35 mm in diameter, gap 1.0 mm). Starch suspension (30 and 40%, w/w) were prepared in test tubes with screw caps so that total sample size was constant at 2.4 g. The suspension was stirred continuously at 500 rpm for 30 min at room temperature using a magnetic stirrer, and heated in a water bath at 55°C for 2.5–4.5 min with continuous stirring at 500 rpm until the suspension became thick enough to prevent starch settling. Pastes were transferred between two glass plates with a 1.0-mm spacer and sealed in airtight bags, which were heated at 100°C for 15 min. After cooling at 25°C for 5 min, starch gels were stored at 5°C for 1, 8, 16, 24, and 48 hr. For rheological measurement, a 35-mm diameter disk was cut from the center of the gel and transferred to the rheometer plate. Dynamic viscoelasticity was measured in a frequency range of 0.01–10 Hz at 25°C and constant stress (50 Pa). At this stress, all samples showed linear behavior. Silicone oil was applied to the exposed surfaces of the sample to prevent evaporation during the experiment.

### Statistical Analysis

All samples were analyzed in duplicate. Dynamic viscoelastic parameters were measured at least in triplicate. The general linear model (SAS Institute, Cary, NC) was used to analyze data. Analysis of variance was conducted using Tukey's studentized range test at the 5% significance level.

## RESULTS AND DISCUSSION

### Amylose Content and Amylopectin Structure

The amylose content of waxy wheat starch was 1.4–1.7%, and that of nonwaxy starch 18.5–28.6% (Fig. 1). The two waxy wheat lines had no waxy protein (Kiribuchi-Otobe 1997; Yasui et al 1997), and the four low-amylose lines are Wx-A1 and Wx-B1 protein-deficient. Within low-amylose lines, Kanto 122 showed lower amylose content than other low-amylose lines. Cadoux and Eradu, which are Wx-B1 protein-deficient (Zhao et al 1998), showed amylose contents similar to that of Norin 61 and Bandouwase with three waxy proteins. It was reported that the lack of one waxy protein reduces amylose content in starch (Yamamori et al 1992, 2000; Naka-

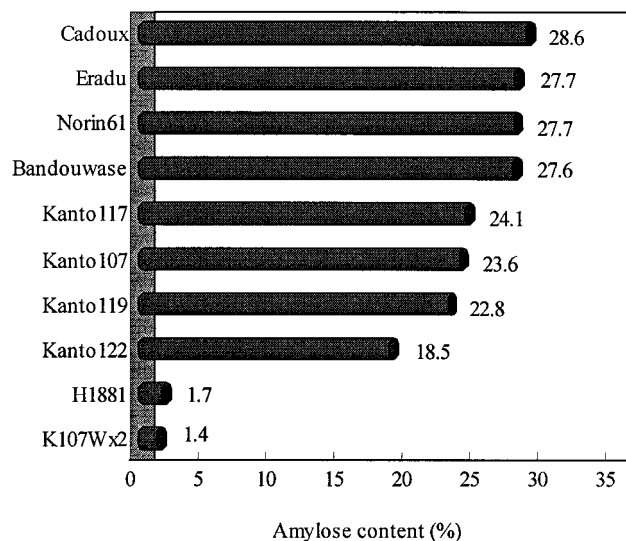


Fig. 1. Amylose content of isolated starch.

mura et al 1993). However, the amylose content of starch is also affected by environment. In this study, only Cadoux and Eradu were sheltered before harvesting to prevent sprouting, which suggests that environmental differences during maturation may have increased the amylose content of Cadoux and Eradu.

Table I shows the chain-length distribution of amylopectin. The peak area ratio of DP 6–12 was 24.4–25.7%, that of DP 13–34 was 57.7–58.4%, and that of DP  $\geq$  35 was 16.2–17.9%, and the variation of peak area ratio was narrow. Waxy lines contained higher proportions of longer chains (DP  $\geq$  35) compared with other lines, and starches with higher amylose content tended to have higher proportions of short chains (DP 6–12). The gelling behavior of starch was influenced by the fine structure of amylopectin (Hoover 1995). However, no marked difference in chain-length distribution of amylopectin was found in these starches.

### Swelling Power, Solubilized Starch and Amylose

In the measurement of swelling power and solubilized starch, the two waxy wheat lines did not form gels, so it was not possible to separate the phase and analyze swelling power and solubilized starch. The swelling power of nonwaxy starches had a range of 16.7–26.0 g/g (Table II). Amylose content correlated highly with swelling power ( $r = -0.93^{**}$ ). This inverse correlation agreed with the relationship observed by Tester and Morrison (1990, 1992) and Morrison et al (1993). They suggested that starch swelling is a property of amylopectin, and amylose acts as a diluent. Solubilized starch in isolated starches was 10.2–17.7%, while solubilized amylose in starch was 7.3–12.9%, and amylopectin was 2.3–5.0% (Table II). The insoluble amylose fraction in starch was 11.2–17.3%, and amylopectin was 67.5–76.5% (Table II). Low-amylose starches other than Kanto 119 showed lower solubilized starch. Solubilized amylose correlated with amylose content in starches ( $r = 0.85^{**}$ ). Results indicated that more amylose leached out for starches with high-amylose content, and starch swelling area decreased. Cadoux and Eradu showed higher swelling power and lower solubilized amylose and amylopectin than Norin 61 and Bandouwase, despite having similar amylose content, suggesting that both amylose and amylopectin molecules in Norin 61 and Bandouwase were more sensitive to leaching out in hot water than Eradu and Cadoux. Insoluble amylopectin correlated highly with swelling power ( $r = 0.98^{**}$ ), indicating that amylopectin solubility was responsible for starch swelling.

### Dynamic Viscoelasticity

The frequency dependence of the storage shear modulus ( $G'$ ) of 30 and 40% wheat starch gels stored at 5°C for 1 hr is shown in Figs. 2 and 3. Both 30 and 40% low-amylose starch gels exhibited lower  $G'$  than starches with high-amylose content through 48 hr of storage. Waxy starch gels were very soft and showed significantly lower  $G'$  compared with nonwaxy starches. For low-amylose starches, Kanto 122 showed significantly lower  $G'$  than the other

TABLE I  
Chain Length Distribution of Amylopectin

Sample	Chain Length Distribution <sup>a,b</sup>		
	DP 6–12	DP 13–34	DP $\geq$ 35
Cadoux	25.2ab	58.4a	16.4bc
Eradu	25.0ab	58.3a	16.7a-c
Norin 61	25.6a	58.0a	16.4bc
Bandouwase	25.7a	58.2a	16.2c
Kanto 117	24.7b	58.0a	17.4a-c
Kanto 107	24.9ab	58.2a	16.9a-c
Kanto 119	24.9ab	58.1a	17.0a-c
Kanto 122	24.6b	58.3a	17.2a-c
H1881	24.5b	57.7a	17.9a
K107Wx2	24.4b	58.0a	17.7ab

<sup>a</sup> Sum of peak-area ratios (%) of group.

<sup>b</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

low-amylose starches (Kanto 107, Kanto 117, and Kanto 119). When heated in the presence of water, breakdown of the amylopectin matrix severely weakens the granule structure and the disentanglement of amylopectin chains results in softer starch gels (Keetles et al 1996a). Hermansson and Svegmak (1996) and Mei-Lin et al (1997) reported that amylose helps reduce loss in the granular rigidity of swollen starch granules. Results thus indicate that amylose plays a major role in forming a firm gel structure, and that the slight difference in amylose content contributes greatly to the elastic component in starches forming a gel network. Cadoux and Eradu formed a slightly softer gel during storage than Norin 61 and Bandouwase, despite having a similar amylose content. As shown in the results for solubilized starch measurement (Table II), amylose in Norin 61 and Bandouwase was more sensitive to leaching out during heating than Cadoux and Eradu. Orford et al (1987) reported that reducing the amount of solubilized amylose would lead to a softer gel. Consequently, more leached-out amylose formed a gel network in Norin 61 and Bandouwase starches, which resulted in a harder gel than Cadoux and Eradu. For 30 and 40% starch gels of nonwaxy wheat lines,  $G'$  exhibited very little frequency dependence over the frequency range (0.01–10 Hz) during storage (Fig. 2). The very low frequency dependence of starch gels means a typical gel with permanent cross-links (Biliaderis and Tonogai 1991), which indicates that nonwaxy starch gels formed a network

structure even after 1 hr of storage at 5°C. For nonwaxy starches, 30 or 40% was concentrated enough to form a gel network in the initial stage of storage at 5°C. Waxy starch gels showed higher frequency dependence than other starches (Fig. 3), with that of 30% waxy starch gels higher than that of 40% waxy starch gels. The frequency dependence of 30 and 40% waxy starch was depressed with increasing storage duration at 5°C, meaning a network structure was continuously formed in waxy starch gels during storage.

Figures 4 and 5 showed changes in  $G'$  of 30 and 40% wheat starch gels during storage at 5°C. The storage shear modulus of all starches continuously increased during storage (48 hr). This increase in  $G'$  results from the rearrangements involving amylose and amylopectin (Keetles et al 1996a). The increase in modulus during the first day of storage is more pronounced for the 40% starch gel than for 30%. Starch with higher amylose content showed a high initial  $G'$ , indicating a more rapid gel network formation compared with starch with lower amylose content. Nonwaxy starches suppressed the development of  $G'$  after 24 hr, while waxy starch developed the gel network steadily after 24 hr. The  $G'$  of 40% waxy starch in particular increased steeply, approaching that of other starches after 48 hr. Long-term changes associated with retrogradation are attributed to the recrystallization of amylopectin fraction, and short-term changes are attributable to the amylose fraction causing

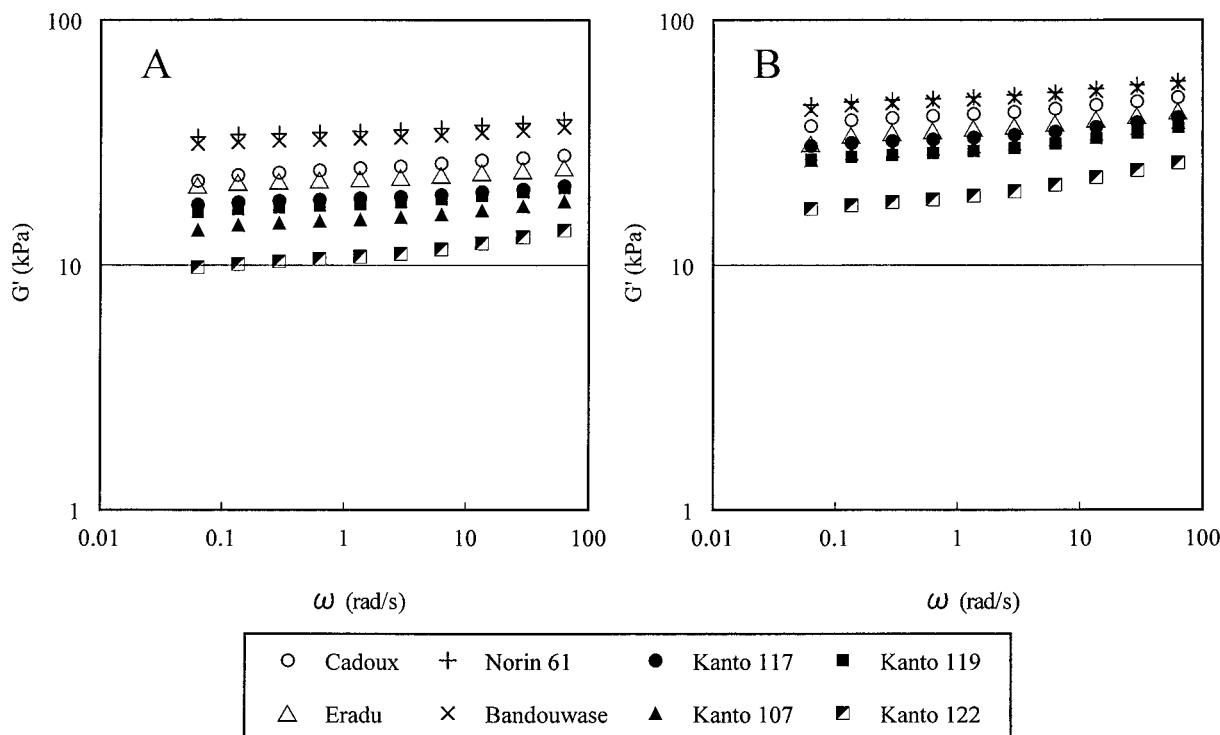


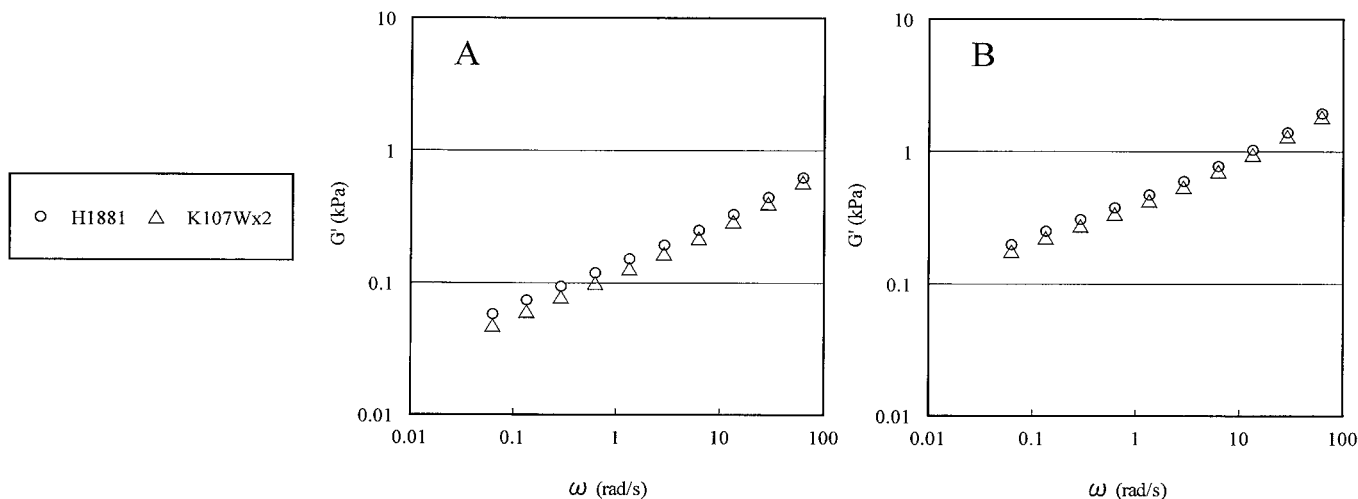
Fig. 2. Frequency dependence of storage shear modulus ( $G'$ ) of nonwaxy starch gels stored at 5°C for 1 hr. A = 30% (w/w); B = 40% (w/w).

TABLE II  
Swelling Power and Solubilized and Insoluble Starch for Nonwaxy Starches<sup>a,b</sup>

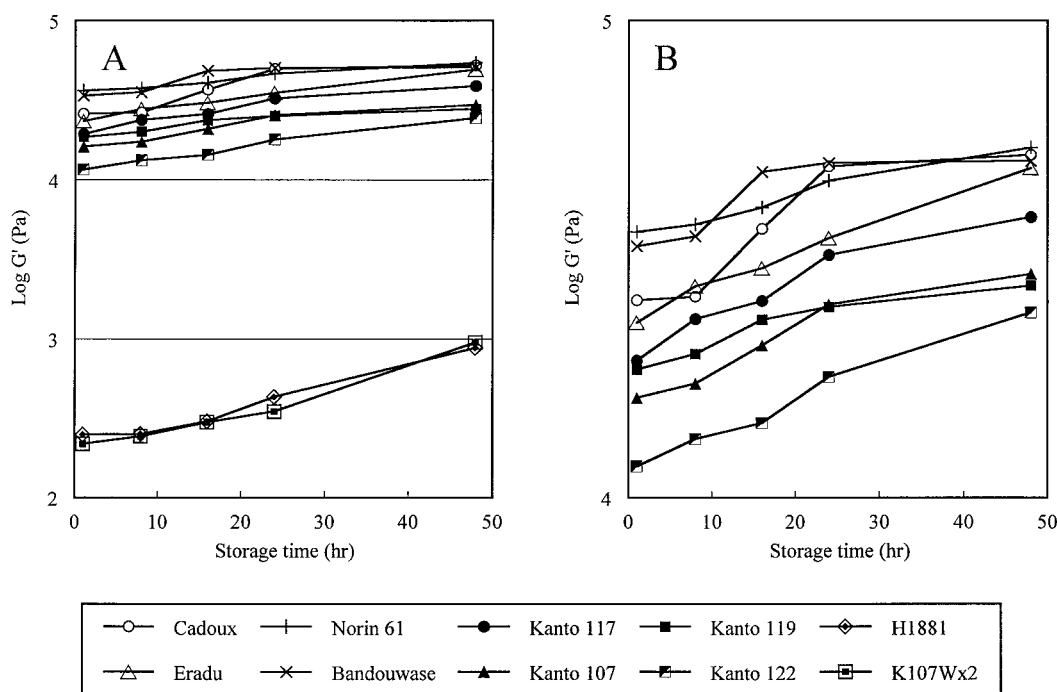
Sample Starch	Swelling Power (g/g)	Solubilized Starch (%)	Solubilized Amylose (%)	Solubilized Amylopectin (%)	Insoluble Amylose (%)	Insoluble Amylopectin (%)
Cadoux	18.6b	14.6bc	11.7b	2.9b–d	16.9a	68.5cd
Eradu	19.6b	12.8c	10.4c	2.3d	17.3a	69.9c
Norin 61	16.8a	17.7a	12.9a	4.8a	14.8c	67.5d
Bandouwase	16.7a	16.9ab	12.9a	4.0a–c	14.8c	68.4d
Kanto 117	22.6c	12.0c	9.3d	2.8b–d	14.8c	73.1b
Kanto 107	22.8cd	10.2d	7.8e	2.4cd	15.8b	73.9b
Kanto 119	23.9d	14.1c	9.9cd	4.1ab	12.9d	73.0b
Kanto 122	26.0e	12.3c	7.3e	5.0a	11.2e	76.5a

<sup>a</sup> Dry-weight percentage of original starch.

<sup>b</sup> Values followed by the same letter are not significantly different ( $P < 0.05$ ).



**Fig. 3.** Frequency dependence of storage shear modulus ( $G'$ ) of waxy starch gels stored at 5°C for 1 hr. **A** = 30% (w/w); **B** = 40% (w/w).



**Fig. 4.** Changes in storage shear modulus ( $G'$ ) at a frequency of 1 Hz of 30% wheat starch gels with storage period at 5°C. **A** = all isolated starch; **B** = nonwaxy starch.

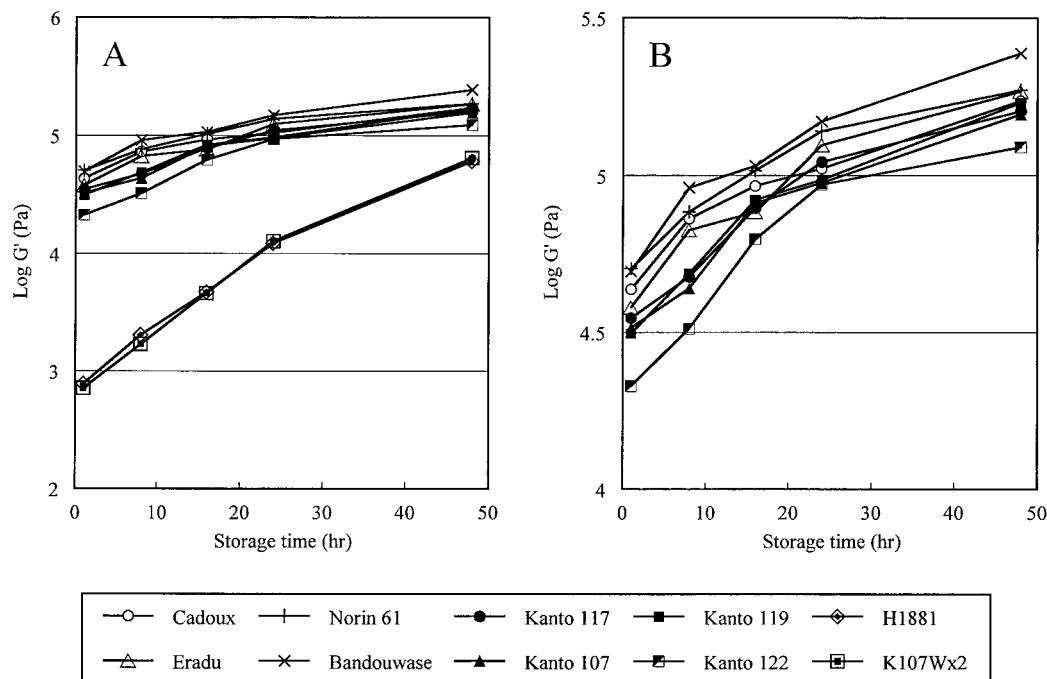
initial gel network formation (Gudmundsson 1994). The long-term reordering of amylopectin is reported to be slow, involving recrystallization of the outer branches of amylopectin and increasing the rigidity of the swollen granules, thus reinforcing the continuous amylose phase (Abd Karim et al 2000, Ring 1987). In this study, nonwaxy starch gels were considered to initially and rapidly form a firm network by reassociation of amylose and increased the rigidity of the gel over 48 hr by recrystallization of amylopectin. In contrast, waxy starch gel continuously increased rigidity due to the reordering of amylopectin.

The retrogradation process of starch gels in the initial stage within 24 hr may be expressed by the first-order kinetic equation:

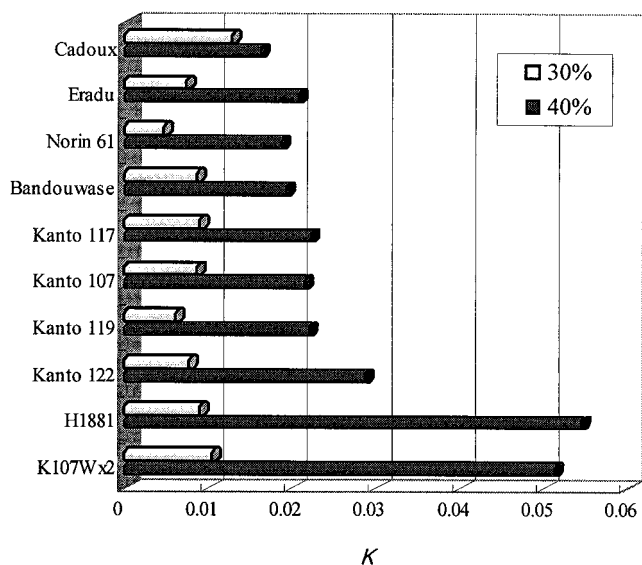
$$\text{Log } G' = kt + C$$

where  $k$  = the rate constant,  $t$  = storage time, and  $C = \text{Log } G'$  when storage time is 0 (Katsuta et al 1992). Figure 6 compares the rate constant of 30 and 40% starch gels. For 30% starch gels, the rate constant was similar and showed no clear relationship to amylose content in any starch used. However, for 40% starch gels, the rate

constant of low-amylose starches was higher than that of starches with high-amylose content. Waxy starch gels at 40% exhibited particularly greater retrogradation rates, and the considerable difference between 30 and 40% waxy starch gels suggests that water content affected the reassociation of amylopectin and the extent of retrogradation of starch gels. Because starch concentrations of suspension are high, the swelling area of individual granules is restricted when heated and swollen granules become tightly packed (Keetles et al 1996b,c). In a low-starch concentration, swollen granules cannot occupy available area, and amylose easily leaches out of granules during heating, forming an amylose gel layer after cooling (Flipse et al 1996). In a concentrated system, as in this study, amylose leaching was considered to be suppressed by the surrounding area occupied by swollen granules, and the amylose matrix between swollen granules became very small. This suggests that recrystallization of the outer branches of amylopectin in the swollen area played a major role in retrogradation of the starch gels during storage, resulting in waxy starch or lower amylose starch with a greater retrogradation rate using a 40% suspension.



**Fig. 5.** Changes in storage shear modulus ( $G'$ ) at a frequency of 1 Hz of 40% wheat starch gels with storage period at 5°C. **A** = all isolated starch; **B** = nonwaxy starch.



**Fig. 6.** Rate constant of developing storage shear modulus in the initial stage (24 hr) at 5°C.  $k$  = rate constant.

## CONCLUSIONS

The difference in amylose content between the lines used in this study greatly influenced starch swelling, solubility, and gel properties. Lower amylose starches showed higher swelling power and lower solubilized amylose. Concentrated gels of low-amylose starches were softer than starches with high-amylose content during 48 hr of storage. Waxy starch gels showed characteristic gel properties. The storage shear modulus ( $G'$ ) of waxy starch gels was markedly low, and the frequency dependence was high, indicating that the gel network structure in waxy starch gel was weak. In 40% starch gels, low-amylose starches showed faster development of  $G'$  than starches with high-amylose content, and waxy starch showed very steep development of  $G'$ . These results suggest that the reassociation of amylopectin plays a major role in the retrogradation of the concentrated starch system.

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