

## REVIEW

# Effect of Wheat Starch Characteristics on the Gelatinization, Retrogradation, and Gelation Properties

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### Abstract

The objective of this study was to clarify the relationship between amylose content and the gelatinization, retrogradation, and gelation properties of starch using wheat starches differing in amylose content. Starches were prepared by reciprocal crossing between waxy and non-waxy wheat, and mixing waxy and non-waxy wheat starch. Gelatinization temperature and endothermic enthalpy measured by differential scanning calorimetry correlated negatively with amylose content. Mixed starches showed significantly lower gelatinization onset and peak temperature than F<sub>1</sub> starches with the same amylose content. Rapid Visco Analyser measurement showed that F<sub>1</sub> starches had a higher peak viscosity than waxy and non-waxy wheat starches, and mixed starches had characteristic profiles with two low peaks. The rheological properties of 30% and 40% starch gels were measured using mixed starches with an amylose content of 5, 10, 18, 20, 23, and 25%. The slight difference in amylose content contributed greatly to the elastic component in starches forming a gel network. For 40% starch gels, mixed starch with lower amylose rapidly developed rigidity and had a higher rate constant of starch retrogradation. The proportion of waxy starch in the mixture played a major role in starch gel properties. Some of the gelatinization and gelation properties differed between mixed starch and simple starch isolated from cultivars, even though they have similar amylose content.

**Discipline:** Agricultural chemicals

**Additional key words:** amylose content, starch gel, waxy wheat

### Introduction

Starch is the major constituent of wheat (*Triticum aestivum* L.) endosperm and an important structural component in many food products made from wheat flour. 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. 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<sup>14</sup>. These changes in starch structure, known as retrogradation, decrease the eating quality of starch-based products<sup>1,10</sup>. The amylose content greatly influences the physicochemical properties

of starch, such as gelatinization, retrogradation, and gelation<sup>3,7,17,27</sup>. Amylose synthesis depends mainly on the waxy protein, granule-bound starch synthase (GBSS)<sup>4,22</sup>. Several waxy wheats lacking three waxy proteins, Wx-A1, Wx-B1, and Wx-D1, were produced by cross-breeding or mutation<sup>11,15,25,26</sup>. Moreover, several low-amylose cultivars lacking two waxy proteins (Wx-A1 and Wx-B1) have been released in recent years. Because of these developments, wheat starch with different amylose content is available. However, the distribution of amylose content in wheat starch is not so wide. In this study, we produced starch with varied amylose content not found in existing cultivars by reciprocal crossing between waxy and non-waxy wheat and prepared mixed starch by blending waxy and non-waxy wheat starch at various ratios. We compared the gelatinization, retrogradation, and gelation properties of starch with widely differing amylose content, and also analyzed the effect of amylose content on the physicochemical properties of starch.

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## Materials and methods

### 1. Samples

Six waxy wheat lines (Wx-1, Wx-2, Wx-3, Wx-4, K107Wx1, and K107Wx2) and two non-waxy parent wheats (Kanto 107 and Saikai 173) were used for reciprocal crossing. Wx-1 and Wx-2 were derived from a cross between Bui-Huo (Wx-D1 protein-deficient cultivar) and Saikai 173 (Wx-A1 and Wx-B1 protein-deficient cultivar). Wx-3 and Wx-4 were derived from a cross between Bui-Huo and Kanto 107 (Wx-A1 and Wx-B1 protein-deficient cultivar). K107Wx1 and K107Wx2 were mutants of Kanto 107 with ethyl methanesulphonate. Reciprocal crossing between waxy wheats and their double-null non-waxy parent yielded two types of F<sub>1</sub> seeds (waxy/non-waxy and non-waxy/waxy). For purpose of comparison, mixed starches were produced by blending waxy and non-waxy starch at different ratios determined to correspond to the amylose content of F<sub>1</sub> seeds.

For evaluating the rheological properties of concentrated starch gels, a waxy line, K107Wx1, and a normal amylose line, Norin 61, were used to isolate starches. Mixed starches were prepared by blending waxy and non-waxy starches at different ratios to yield an amylose content of 5, 10, 18, 20, 23, and 25%. Thirty percent and 40% (w/w) starch gels were prepared for analyzing dynamic viscoelasticity.

Amylose content of starch was determined using an amylopectin/amylose assay kit (Megazyme International Ireland Ltd., Ireland).

### 2. Differential scanning calorimetry measurement

Differential scanning calorimetry (DSC) measurement was conducted using a SSC 5200 with a DSC 120U (Seiko Electronics, Tokyo, Japan) calibrated with indium. For gelatinization studies, 15.0 mg of starch was weighed into silver pans with 35 µL of distilled water. After sealing, pans were scanned at 1°C/min from 40 to 120°C. After gelatinization, pans were immediately cooled and stored at 5°C for 4 weeks and rescanned at 1°C/min from 5 to 120°C for retrogradation studies.

### 3. Starch pasting properties

The pasting properties of isolated starch were measured with a Rapid Visco Analyser (RVA, Newport Scientific Pt. Ltd., Warriewood, Australia). Starch, 1.5 g (db), was dispersed in 12.5 mL of distilled water. The suspension was heated at 5°C/min from 34 to 94°C, held at 94°C for 5 min, and cooled at 5°C/min to 34°C and held at 34°C for 10 min.

### 4. Rheological measurement

The dynamic viscoelasticity of starch gel was measured using a rheometer (RheoStress RS75, HAAKE, Germany) with a parallel plate (35 mm diameter, gap 1.0 mm). For rheological measurement, a 35 mm diameter disk was cut from the center of the gel and transferred on the rheometer plate. The linear viscoelastic region was determined by means of a stress sweep. 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 exposed surfaces of starch gels to prevent evaporation of water during the experiment.

## Results and discussion

### 1. Comparison of starch characteristics of waxy, non-waxy, their F<sub>1</sub> seeds, and mixed starch

#### (1) Amylose content

The amylose content of waxy wheat lines was 0.8–0.9%, and that of non-waxy wheat 18.3–20.3%, whereas that of F<sub>1</sub> seeds was 7.2–7.7% (waxy/non-waxy) and 13.5–15.3% (non-waxy/waxy) (Table 1). F<sub>1</sub> seed starch had an amylose content not found in existing cultivars and ranging between waxy and non-waxy wheat<sup>18</sup>. None of the six waxy wheats had waxy protein<sup>15,25,26</sup>. All F<sub>1</sub> seeds were derived from the cross between Wx-A1, Wx-B1, and Wx-D1 protein-deficient cultivars and Wx-A1 and Wx-B1 deficient cultivars. The difference in amylose content between waxy/non-waxy and non-waxy/waxy starch in F<sub>1</sub> seeds suggests that gene dosage has effects on amylose content by double fertilization (Table 1).

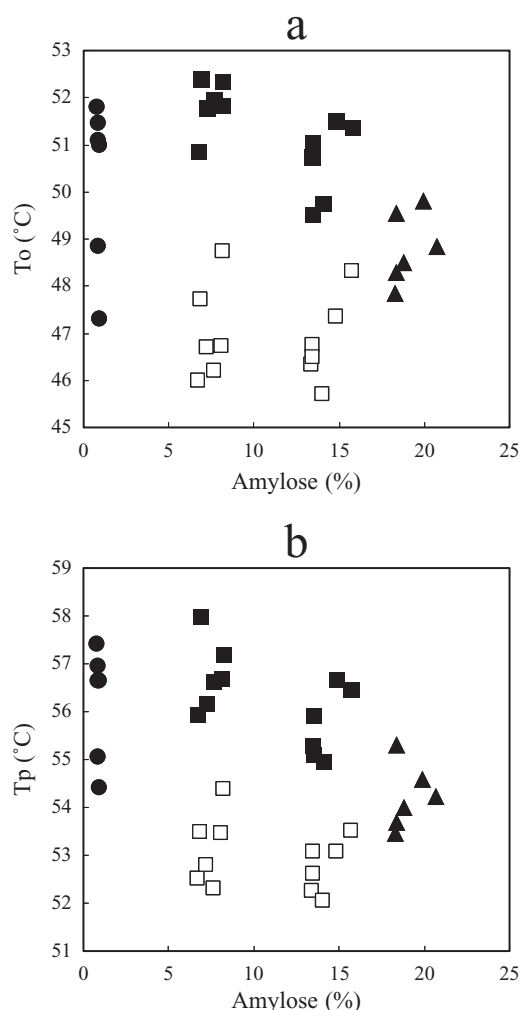
**Table 1. Dosage effect on amylose content in endosperm**

Sample	Functional <i>Wx-D1</i> allele dosage in endosperm	Amylose content (%)
Non-waxy	3	18.3–20.3
Non-waxy/Waxy	2	13.5–15.3
Waxy/Non-waxy	1	7.2– 7.7
Waxy	0	0.8– 0.9

## (2) Gelatinization and Retrogradation Properties

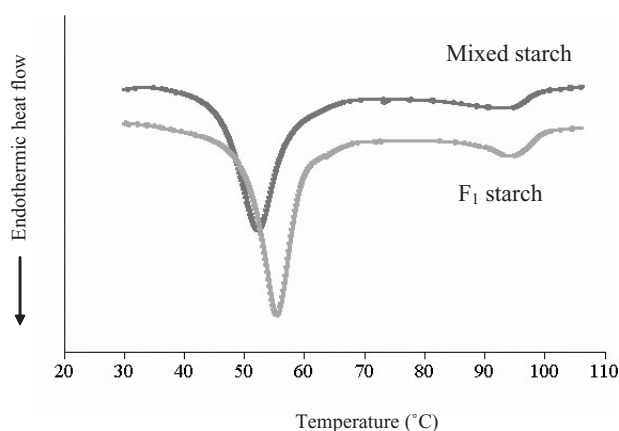
Differential scanning calorimetry (DSC) measurement was conducted for gelatinization and retrogradation studies. Final gelatinization temperature and gelatinization enthalpy correlated negatively with amylose content in all samples ( $r = -0.80^{**}$ ,  $-0.87^{**}$ )<sup>18</sup>. No significant correlations were found between amylose content and onset and peak gelatinization temperature.  $F_1$  and mixed starches having the same amylose content as  $F_1$  starches showed clearly different gelatinization onset and peak temperature (Fig. 1). Mixed starches showed lower onset and peak temperatures than  $F_1$  starches having the same amylose content. Curves of mixed starch were broader than those of  $F_1$  starch (Fig. 2). The enthalpy of gelatinization reflects the loss of molecular order<sup>2</sup>, and gelatinization temperature is considered a parameter of crystallite perfection<sup>21</sup>. Since amylopectin plays a major role in starch granule crystallinity, the presence of amylose lowers the melting temperature of crystalline regions and the energy for starting gelatinization<sup>6</sup>. More energy is needed to initiate melting in the absence of amylose-rich amorphous regions<sup>12</sup>. The correlation indicates that starch with higher amylose content has more amorphous region and less crystalline, lowering gelatinization temperature and endothermic enthalpy. The differences in gelatinization properties between  $F_1$  and mixed starches are due to varied homogeneity. Fredriksson et al.<sup>7</sup> suggested that a wide temperature range implied a large amount of crystals with varied stability. The heterogeneity of ordered inside structures causes a broader gelatinization range. We found the gelatinization range of mixed starches broader than  $F_1$  starch having the same amylose content, indicating that the crystallite stability of mixed starch differs from that of  $F_1$  starch having the same amylose content. Starch granule crystallites that required less energy to melt would melt first<sup>16</sup>. Non-waxy starch with higher amylose content would start to melt first, and waxy starch melted successively in mixed samples, which results in lowering gelatinization onset and peak temperature of mixed starches. Another endothermic peak was observed at about 100°C in non-waxy,  $F_1$ , and mixed starches, but no corresponding peak was found in waxy wheat starch. This peak is thought to correspond to transition of the amylose-lipid complex<sup>5</sup>. Endothermic enthalpy due to the melting of amylose-lipid complexes correlated significantly with amylose content ( $r = 0.88^{**}$ ), as found elsewhere<sup>24</sup>.

Gelatinization temperature and enthalpy in retrograded starches were analyzed after storage at 5°C for 4 weeks. Retrograded starches showed lower gelatinization temperature and smaller enthalpy than raw starches, indicating that they have weaker starch crystallinity. The



**Fig. 1. Relationship between amylose content and gelatinization temperature**

a: Onset temperature ( $T_o$ ), b: Peak temperature ( $T_p$ ).  
●: Waxy, ■:  $F_1$ , ▲: Non-waxy, □: Mixed.



**Fig. 2. DSC (differential scanning calorimetry) profiles (30%) of  $F_1$  starch from a cross between Kanto 107 and K107Wx1, and mixed starch with the same amylose content as  $F_1$  starch**

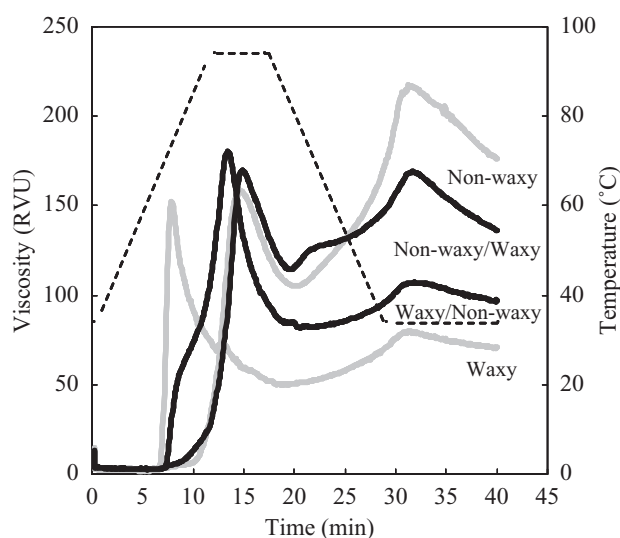
enthalpy for melting recrystallized starches correlated negatively with amylose content ( $r = -0.55^*$ ), showing the starch with more amylopectin recrystallized to a greater degree. Miles et al.<sup>14</sup> reported that the amylose component in starch gels retrogrades rapidly and the crystallization of amylose reached a limit after 2 days, whereas the amylopectin component recrystallizes slowly over time. After a few days of storage, the amylopectin is highly crystallized and long-term changes in crystallization are mainly associated with the amylopectin fraction<sup>8</sup>. Since starches with higher amylopectin content tend to recrystallize more during a longer storage, higher energy is needed to melt the reformed crystallite. No difference in retrogradation properties was found between  $F_1$  and mixed starch.

### (3) Pasting Properties

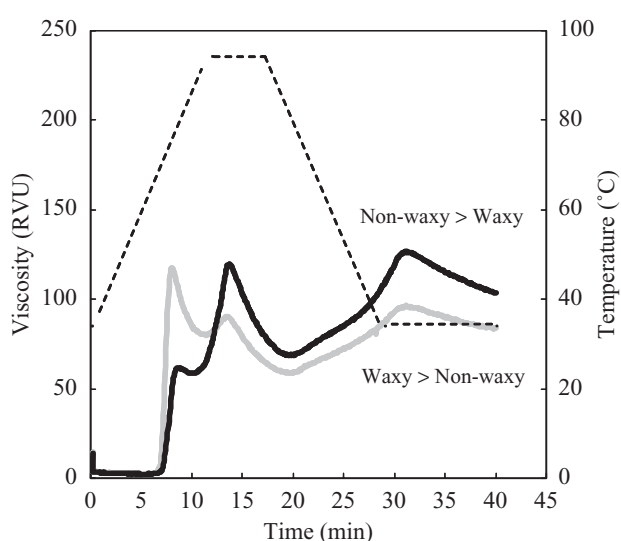
The pasting properties of isolated starch were measured with a Rapid Visco Analyser (RVA).  $F_1$  starch had higher peak viscosity than non-waxy wheat starch (Fig. 3), supporting the idea that lower amylose content is associated with higher peak viscosity. Reduced amylose-content starch relates to greater swelling. Greater swelling reduces the quantity of free water and is associated with higher pasting viscosity<sup>28</sup>. In this study, waxy starch showed a lower peak viscosity than  $F_1$  starch and a very sharp increase in paste viscosity at lower temperature (Fig. 3). Leloup et al.<sup>13</sup> demonstrated that a minimum amylose:amylopectin ratio of 0.43 was needed to maintain the gel network during heating in water. At lower amylose content, the structure of starch gel is easily dis-

rupted by heating. Amylose suppresses swelling and maintains the integrity of swollen starch granules<sup>9</sup>. Since starch swelling is mainly a property of amylopectin, waxy starch swells rapidly and swollen granules degrade at lower temperature, indicating that waxy starch rapidly develops viscosity but cannot maintain the stability of paste viscosity. For  $F_1$  starches, a clearly different step in increasing viscosity was found between non-waxy/waxy and waxy/non-waxy. Non-waxy/waxy starch showed a profile similar in increasing viscosity to non-waxy starch. Waxy/non-waxy starch, however, showed a sharp increase in viscosity at lower temperature, similar to the profile of waxy starch. This difference would reflect amylose content.

Mixed starch showed characteristic RVA profiles having two low peaks (Fig. 4). The peak viscosity of mixed starches was much lower than that of waxy and non-waxy wheat and their  $F_1$  starch. In mixed starch including more waxy starch, the first peak was higher than the second, and in mixed starch including more non-waxy starch, the second peak was higher. The two peaks indicate that waxy starch in the mixed sample started to collapse before non-waxy starch reached the peak viscosity. When waxy starch granules broke down, non-waxy starch granules developed a viscosity. The difference in pasting properties between waxy and non-waxy starch produced two low peaks in the pasting profile of mixed starch. Results also indicate that the association between amylose and amylopectin molecules in mixed starch would differ from that of simple starch. Mixed starch may induce specific starch chain interactions between



**Fig. 3. Starch pasting curves of waxy and non-waxy wheat and their  $F_1$  seeds**  
 ----- : Temperature profile.



**Fig. 4. Starch pasting curves of mixed starches of waxy and non-waxy wheat**  
 ----- : Temperature profile.

molecules, starch granules, swollen granules, and granule fragments.

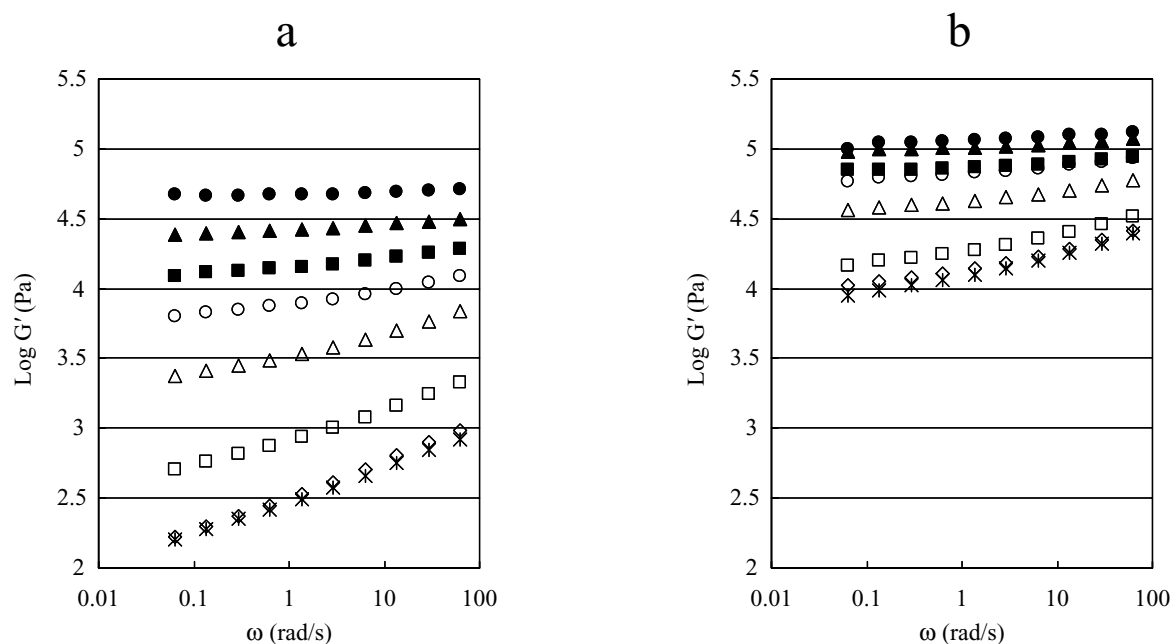
## 2. Rheological properties of mixed starch gels

Evaluation of concentrated starch gel provides useful information on food quality, since the starch concentration is high in wheat products. We compared the rheological properties of mixed starch gels with varied amylose content, and analyzed the effect of amylose content on concentrated starch gel properties<sup>19</sup>.

The frequency dependence of the storage shear modulus ( $G'$ ) of 30% and 40% wheat starch gels stored at 5°C for 24 h are shown in Fig. 5. Both 30% and 40% starch gels mixed with more waxy starch showed lower  $G'$  during 48 h storage, meaning softer gel and weaker gel structure. The small difference in the mixing ratio of waxy starch markedly affected the elastic component of starch gels. The decrease in  $G'$  induced by the increased mixing ratio of waxy starch was more pronounced for 30% than 40% starch gels. Mixed starch gels with 18, 23, and 25% amylose content showed a significantly lower  $G'$  than starch gels prepared from cultivars having a corresponding amylose content of mixed starches<sup>20</sup>. When starch granules are heated with water and gelatinized, amylopectin matrix breakdown severely weakens the granule structure<sup>10</sup>. On the other hand, amylose is reported to help reduce granular rigidity loss of swollen

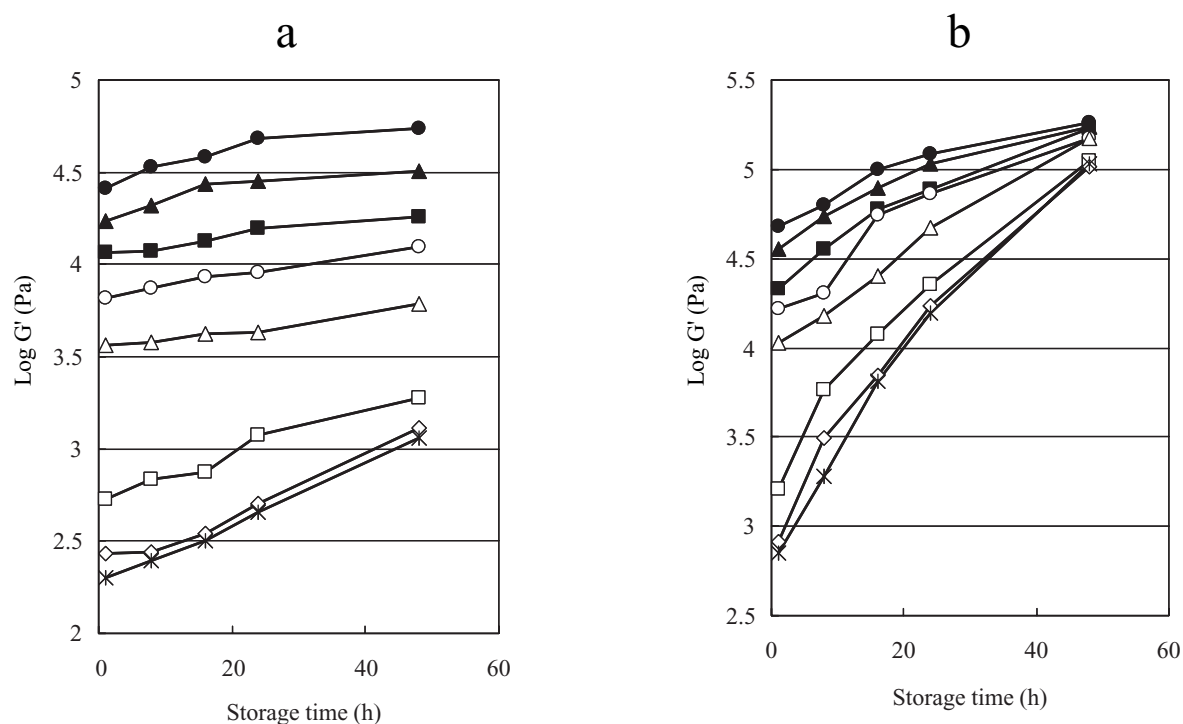
starch<sup>23</sup>, and leached-out amylose plays a role in forming the gel network. The mixed starch with more waxy starch had higher solubilized amylopectin and lower solubilized amylose. Results indicate that mixed starch gel from samples containing more waxy starch leads to a softer and weaker gel than non-waxy starch gel, probably due to increased leached-out amylopectin and reduced leached-out amylose to form the gel network. The frequency dependence of 30% and 40% starch gels decreased with increasing non-waxy starch proportion of mixed starch and increasing storage duration at 5°C. For 40% starch gels, mixed starch even with low amylose content showed a lower frequency dependence, indicating that 40% is a sufficient concentration for mixed starch having low amylose to form a gel network with cross-links.

In  $G'$  development in 30% and 40% starch gels during 48 h storage at 5°C,  $G'$  continued to increase through storage (Fig. 6). In particular, during the initial 24 h of storage the 40% starch gels showed a great increase in  $G'$ . Starch gels of mixed samples with more waxy starch exhibited lower  $G'$  through storage, indicating waxy starch decreased gel network formation. 40% starch gels with less than 10% amylose content, however, developed the gel network rapidly and approached the  $G'$  of other starch gels with higher amylose content after 48 h. The retrogradation of starch gels in the initial stage within 24



**Fig. 5. Frequency dependence of storage shear modulus ( $G'$ ) of 30% (a) and 40% (b) wheat starch gels stored at 5°C for 24 h**

●: Norin 61, ▲: amylose 25%, ■: amylose 23%, ○: amylose 20%,  
△: amylose 18%, □: amylose 10%, ◇: amylose 5%, \*: K107Wx1.



**Fig. 6.** Changes in storage shear modulus ( $G'$ ) at a frequency of 1Hz of 30% (a) and 40% (b) wheat starch gels with storage period at 5°C

●: Norin 61, ▲: amylose 25%, ■: amylose 23%, ○: amylose 20%,  
△: amylose 18%, □: amylose 10%, ◇: amylose 5%, \*: K107Wx1.

**Table 2.** The rate constant of developing storage shear modulus of starch gels

Sample	30% starch gels $k \times 10^{-3} (\text{h}^{-1})$	40% starch gels $k \times 10^{-3} (\text{h}^{-1})$
Norin 61	11.3	18.4
Amylose 25%	9.9	20.4
Amylose 23%	6.1	24.5
Amylose 20%	6.3	30.9
Amylose 18%	3.5	28.2
Amylose 10%	14.4	48.4
Amylose 5%	12.1	55.7
K107Wx1	15.5	59.5

h could be expressed by the following first-order kinetic equation:

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

where  $k$  ( $\text{h}^{-1}$ ) is the rate constant,  $t$  the storage time (h), and  $C = \text{Log } G'$  when storage time is zero. Table 2 shows the comparison between the rate constant of 30% and 40% starch gels. For 30%, mixed starch with 5 and 10% amylose content and waxy starch showed a slightly higher rate constant than other starches. The rate constant of 30% starch gels showed no clear relationship to

the composition of waxy starch in mixed starches. For 40% starch gels, mixed starch with more waxy starch showed a higher rate constant, indicating the content of waxy starch in mixed starch dynamically affects the retrogradation rate of 40% starch gels. The differences found between 30% and 40% starch gels suggest that water content markedly affected the rate of starch retrogradation, in particular, amylopectin reassociation. In a concentrated system such as a 40% starch suspension, swollen granules enriched with amylopectin occupy the available area of suspension, decreasing the surrounding

area and decreasing leached-out amylose. This indicates that the recrystallization of amylopectin in the swollen area accompanying the reassociation of outer branches of amylopectin had a greater effect on the retrogradation of starch gels than the gelation of leached-out amylose, so mixing waxy starch in starch gels increased the retrogradation rate.

## Conclusion

The amylose content varied widely in the isolated starches from waxy wheat, non-waxy wheat, and their F<sub>1</sub> seeds by reciprocal crossing. The various relationships between amylose content and physicochemical properties of wheat starch were demonstrated by using F<sub>1</sub> starch and mixed starches, which have amylose content not found in existing cultivars. Gelatinization enthalpy ( $\Delta H$ ) and final gelatinization temperature (T<sub>c</sub>) measured by DSC correlated negatively with amylose content. For onset (T<sub>o</sub>) and peak (T<sub>p</sub>) gelatinization temperature, mixed starches showed a clearly different trend from F<sub>1</sub> starches having the same amylose content as mixed starch. Mixed starch had a broader endothermic peak than F<sub>1</sub> starch. The peak viscosity of F<sub>1</sub> starch measured by RVA was higher than that of their parent cultivars, non-waxy and waxy wheat. Mixed starches showed characteristic pasting profiles, which had two low peaks. Small differences in the mixing ratio of waxy starch markedly affected mixed starch gel properties. The concentration of starch suspension had great influence on the development of modulus. Forty per cent waxy starch gel showed a rapid increase in modulus compared with 30% waxy starch gel, which suggests the concentration affected the reassociation of amylopectin. Mixed starches formed softer gels compared with starches having similar amylose content isolated from a single cultivar. The results suggest that some gelatinization, pasting and gelation properties reflect the homogeneity of starch granules in starch in addition to amylose content. Mixing waxy wheat starch for adjusting amylose content may have a greater impact on the physical properties of starch than expected due to its unique characteristics. When we utilize mixed starches of waxy wheat for food products, it is essential to consider that the heterogeneity of starch granules influences the properties of the final product.

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