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Carbohydrate Research 314 (1998) 221–235

CARBOHYDRATE
RESEARCH

# Effects of moisture content and different gelatinization heating temperatures on retrogradation of waxy-type maize starches

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Received 19 August 1998; accepted 30 October 1998

**Abstract**

The effect of moisture content on retrogradation of *wx*, *du wx*, *ae wx*, and *su2 wx* maize starches was studied by differential scanning calorimetry (DSC). All starches showed maximum retrogradation enthalpy at an intermediate moisture content, but the precise relationship between moisture content and retrogradation enthalpy varied for each starch. The *ae wx* starch was least affected by moisture content, showing measurable retrogradation (2–3 J/g) after only 1 day for the 95% moisture sample. Retrogradation of the *su2 wx* starch was exceptionally slow even at intermediate moisture contents, and the retrogradation enthalpy was particularly sensitive to moisture content. The effect of varied moisture content was also investigated in combination with initial heating to a wide range of temperatures. Retrogradation of *wx*, *du wx*, and *su2 wx* starches, but not *ae wx* starch, was strongly influenced by both initial heating temperature and moisture content, with development of enthalpy consistently in the order of *du wx* > *wx* > *su2 wx*. The chain length profiles for the four starches were unique. The exceptionally rapid retrogradation and relatively small concentration dependence of *ae wx* starch may be related to a greater proportion of longer chains, whereas the very slow retrogradation of *su2 wx* starch may be related to a population of exceptionally short chains. The more rapid retrogradation of *du wx* starch than *wx* starch may be related to the high number and concentration of branch points in a cluster. © 1998 Elsevier Science Ltd. All rights reserved.

*Keywords:* Waxy maize starch; Corn starch; Moisture content; Retrogradation; Gelatinization heat treatment; Chain length

## 1. Introduction

Retrogradation has been used to describe changes in physical behavior following gelatinization. These changes are due to association of starch chains as double helices, and variably ordered semi-crystalline arrays of these helices, as monitored by X-ray diffraction (for crystallinity) or differential scanning calorimetry (DSC) to observe endothermic changes when these structures are lost on heating. Variation in molecular structure of starch can result in altered retrogradation behavior. Structural modification, either by genetic means to change the pathway of starch biosynthesis or by means of chemical or physical modification of starch, has been employed to alter the process of retrogradation. The behavior of starch during retrogradation has been the subject of considerable investigation [1–5]. The retrograded starch, which shows a B-type X-ray diffraction pattern [6], contains

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PII: S0008-6215(98)00310-3

both crystalline and amorphous regions. Because starch retrogradation is a kinetically controlled process [2], the alteration of time, temperature, and water content during processing can produce a variety of end products. After processing, the properties of the metastable starch–water system can also be influenced by moisture content, the botanical source of starch, storage time, and storage temperature [7–9]. At lower water contents, water acting as a plasticizer is well known to affect the $T_g$ (glass transition temperature) of a partially crystalline polymer [10]. Water acts as a plasticizer of amorphous and partially crystalline starch systems. The amount of water affects the glass transition of starch-based foods, and as a consequence it can influence the properties, processing and stability of many starch-based food products [2].

Several studies have investigated starch gelatinization or retrogradation behavior as a function of a wide range of moisture content [11–17]. Zeleznak and Hoseney [8] studied the effect of moisture content on the retrogradation of wheat starch gels, and this work was consistent with that of Longton and LeGrys [7]: maximum crystallization in a wheat starch gel occurred at 40–50% moisture. However, the effect of moisture content on retrogradation of starches with large variation in molecular structure has not been reported.

The structure and functional properties of waxy (*wx*) maize starch have been studied for some time. Other mutations (e.g., *du*, dull; *ae*, amylose-extender; *su2*, sugary 2) in combination with the *wx* mutation produce amylopectin of modified structure [18]. The fine structure of *wx*, *du wx*, and *ae wx* starches was related to retrogradation behavior [19,20]. The chain length distribution of amylopectin appears related to the retrogradation rate of amylopectin in starch [19,20]. Ring et al. [1] suggested that the amylopectin molecules associate by crystallization of the branch chains. After acid hydrolysis and enzymatic debranching of the crystallites, they observed a chain length maximum at degree of polymerization (DP) 15. Yuan et al. [19] proposed that the greater proportion of longer chains in the *ae wx* starch account for the higher enthalpy for both starch gelatinization and retrogradation. De Boer [21] showed that *du wx* starch is more highly branched than *wx* starch, and more recent work [19,20] confirms this observation. From the observed lower gelatinization enthalpy and temperature for *su2 wx* starch, Inouchi et al. [22] assumed that starch containing the *su2* gene had low crystallinity and less hydrogen bonding. More recently, the studies on retrogradation of *wx* and *ae wx* starches [5], and of *du wx* and *su2 wx* starches [23] as a function of gelatinization heating temperature in the presence of excess water (70%, w/w) were reported. The retrogradation of *wx*-containing maize starches was greatly influenced by initial heating to different temperatures. The order of retrogradation enthalpy for these starches after 1 day at 5 °C was $ae\ wx \gg du\ wx > wx > su2\ wx$.

The purpose of the work reported herein was to investigate both the effect of moisture content and the effect of different initial heating temperature on starch retrogradation in the *wx*-type starch–water system in order to better understand the relationship between the structure and properties of amylopectin in maize starches.

## 2. Experimental

*Materials.*—*wx*, *du wx*, and *ae wx* maize starches and common corn starch were gifts from American Maize Products Company (now Cerestar USA, Hammond, IN). *su2 wx* maize starch was a gift from National Starch and Chemical Company (Bridgewater, NJ). The moisture content was measured by AACC method 44-15A, and was found to be 10.8% for *wx* starch, 10.3% for *du wx* starch, 11.6% for *ae wx* starch, 11.3% for *su2 wx* starch, and 10.1% for corn starch.

*Differential scanning calorimetry (DSC).*—Starch was weighed accurately into stainless steel sample pans (319-1605, Perkin–Elmer). Deionized water was added to make starch suspensions with various moisture contents. Total mass of the pan contents was about 30–60 mg, depending on the moisture content. For starch samples of $\leq 70\%$ moisture content, starch was always about 20 mg. For samples of $> 70\%$ moisture content, the total mass was 60 mg. Pans were sealed and equilibrated for 12–24 h at room temperature before heat treatment in the DSC.

Heat treatment of the starch suspensions and the thermal analysis of gelatinization and retrogradation were done using a differential scanning calorimeter (DSC-7, Perkin–Elmer, Norwalk, CT). The DSC was equipped with data analysis software (Pyris for Windows, Perkin–Elmer). Calibration was done with indium. An empty pan was used as a reference.

*Experiment I.* Samples with various moisture contents (20–95% water, w/w) were initially heated at a rate of 10 °C/min from 5 to 140 °C. After heat treatment, samples were quench-cooled to 5 °C. Once the temperature reached 5 °C, samples were immediately removed from the DSC and stored in a refrigerator (5 °C). After 24 h or 7 days, a sample pan was removed from the refrigerator and immediately put into the sample holder of the DSC. Independent samples were prepared for each storage time. Stored samples were heated from 5 to 140 °C at 10 °C/min.

The enthalpy of phase transitions during initial heating was measured from the endotherm of DSC thermograms. Onset temperature ($T_o$), peak temperature ($T_p$), end temperature ($T_e$), and enthalpy ($\Delta H$) for retrogradation endotherms were obtained from the DSC thermograms.

*Experiment II.* Starch samples were made to ~ 50, 65, or 80% moisture content. For all but *ae wx* starch, samples with ~ 65 and 80% moisture content were initially heated at a rate of 10 °C/min from 5 to 85 (just above the completion of the gelatinization endotherm), 140 or 180 °C. The final temperature of the initial heating is referred to as the initial heating temperature. Samples with ~ 50% moisture content were initially heated at a rate of 10 °C/min for all but *ae wx* starch from 5 to 100, 140 and 180 °C. For *ae wx* starch, all the samples were initially heated from 5 to 105, 140 or 180 °C. After heat treatment, samples were quench-cooled to 5 °C. Samples were then immediately removed from the DSC and stored in a refrigerator (5 °C). Separate samples with different initial heating temperature were stored for 24 h or 7 days and were reheated from 5 to 140 °C at 10 °C/min after storage.

*High-performance size-exclusion chromatography (HPSEC).*—HPSEC analysis of debranched starch fractions was done on a system consisting of a pump (6000A solvent delivery system; Waters Division of the Millipore Corp.; Milford, MA) connected in series to an injector (U6K Universal Chromatograph Injector; Waters) and a differential refractometer (model 410 Differential Refractometer, Waters). Two 30-cm columns packed with 6 μm porous silica microspheres (Zorbax PSM 60S, Dupont, Wilmington, DE) connected in series were used for the separations. The columns and detector were maintained at 35 °C. A 50-μL sample was injected into the HPSEC system. The mobile phase in the system was 100% $Me_2SO$ with a flow rate 0.5 mL/min. The chromatograms were analyzed using Millennium Chromatography Manager 2010 software (version 2.15; Waters). The system was calibrated using separate 50-μL injections for each of the following standards: maltotriose (Sigma Chemical Co., St. Louis, MO), maltoheptaose (Sigma Chemical Co.), and three pullulan standards (P-5, P-10 and P-20; molecular weights of $5.8 \times 10^3$, $1.22 \times 10^4$, and $2.37 \times 10^4$, respectively; Showa Danko K.K., obtained through Waters).

Preparation of dispersed-dried starch and debranching of the starch were done based on the method of Klucinec and Thompson [24].

## 3. Results

*Effect of moisture content on starch retrogradation enthalpy.*—The combined enthalpy of the gelatinization and the melting phase transitions at different moisture contents is shown in Fig. 1. The enthalpy varied with the type of *wx*-containing maize starch and the moisture content. In the presence of excess water ( ≥ 70%, w/w), *ae wx* starch had the highest enthalpy among these starches, and *su2 wx* starch had the lowest enthalpy. No difference in enthalpy between *wx* starch and *du wx* starch was apparent. The enthalpy of the combined phase transitions sharply decreased when moisture content dropped below ~ 50% for all starches.

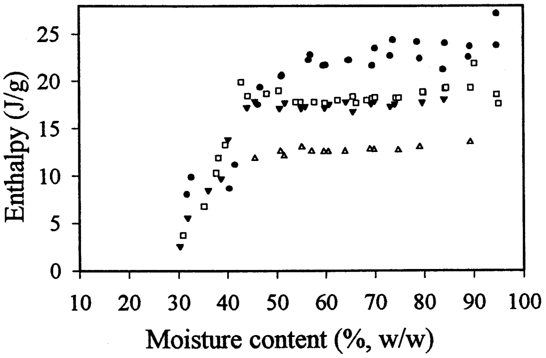


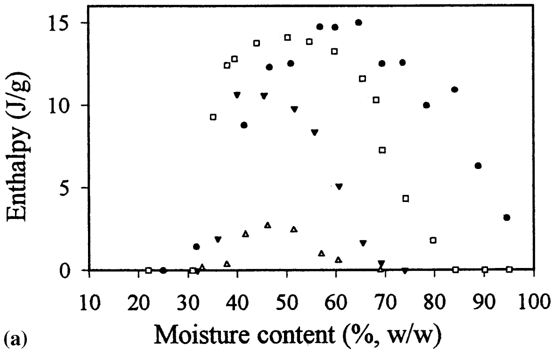
Fig. 1. The combined enthalpy of gelatinization and melting as a function of moisture content during heating to 140 °C for *wx* starch (▼), *du wx* starch (□), *ae wx* starch (●) and *su2 wx* starch (△).

Retrogradation enthalpies for *wx*, *du wx*, *ae wx*, and *su2 wx* starches as a function of moisture content are shown in Fig. 2. DSC thermograms of *du wx* and *ae wx* starches with different moisture content are shown in Figs. 3 and 4, respectively. The *wx* and *su2 wx* starches had thermogram shapes (thermograms not shown) similar to that of *du wx* starch.

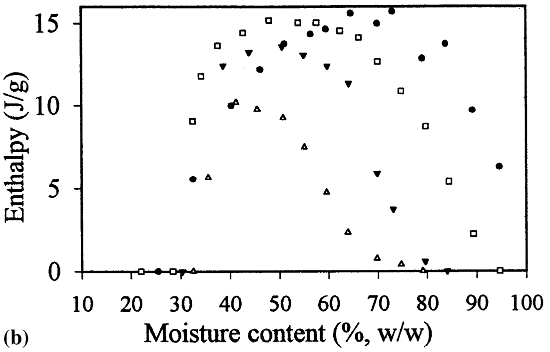
After 24 h at 5 °C, an endotherm for *wx* starch was observed for moisture contents between 35 and 70% (Fig. 2(a)). The maximum of retrogradation enthalpy (about 10 J/g) occurred at moisture content around 45%. A retrogradation endotherm for *du wx* starch was observed after 24 h over a broader range of moisture content (Fig. 2(a)). The maximum enthalpy (about 14 J/g) occurred at moisture content around 50%. For *ae wx* starch after 24 h, retrogradation was observed in all thermograms when moisture content was ≥ 30% (Fig. 2(a)). The maximum of retrogradation enthalpy (about 15 J/g) occurred at moisture content around 65%. For *su2 wx* starch, retrogradation was observed only in the range of 35–60% moisture content (Fig. 2(a)). The maximum of retrogradation enthalpy (2–3 J/g) occurred at moisture content of about 45–50%. The retrogradation enthalpy of *su2 wx* starch was far lower at all moisture contents than other starches at similar moisture contents.

After 7 days, retrogradation enthalpy was higher than for 1-day storage for all starches (Fig. 2(b)). As moisture content increased, retrogradation enthalpy increased up to a maximum value which varied with different starches, but thereafter it decreased as moisture content increased. The maximum retrogradation enthalpy occurred at moisture content of ~ 50% for *wx* starch (13–14 J/g) and for *du wx* starch ( ~ 15 J/g); however, the maximum occurred at ~ 70% moisture for *ae wx* starch ( ~ 16 J/g), but closer to 40% for *su2 wx* starch ( ~ 10 J/g). No retrogradation enthalpy was observed when moisture content was lower than about 30% for any starch. However, at moisture contents > 50%, retrogradation enthalpy varied with moisture content in a different manner for each starch, with the greatest enthalpy at the higher moisture contents in the order *ae wx* > *du wx* > *wx* > *su2 wx* (Fig. 2(b)).

The nature of the retrogradation thermograms for *du wx* starch varied as a function of moisture content. After 24-h storage, the main peak was observed at about 60 °C for 74.2% moisture content, moving to a higher temperature as moisture content decreased. A lower-temperature shoulder was apparent at intermediate moisture content (Fig. 3(a)). On



(a)



(b)

Fig. 2. Retrogradation enthalpy as a function of moisture content for *wx* starch (▼), *du wx* starch (□), *ae wx* starch (●) and *su2 wx* starch (△). (a) After 24 h and (b) after 7 days. Initial heating was to 140 °C (see Fig. 1).

further storage to 7 days, what had been the major peak after 24 h is still evident, but the region of the former lower-temperature shoulder is much enhanced, so much so that it is the main peak at the intermediate moisture contents (Fig. 3(b)).

The nature of retrogradation thermograms for *ae wx* starch was similar in that a main peak and a lower-temperature shoulder were observed. However, the peak temperature for the main peak was higher and its location was less affected by moisture content. After 7-day storage, the magnitude of the low-temperature shoulder was greater than that after 24 h, but in no case did the main peak occur in this region (Fig. 4(b)).

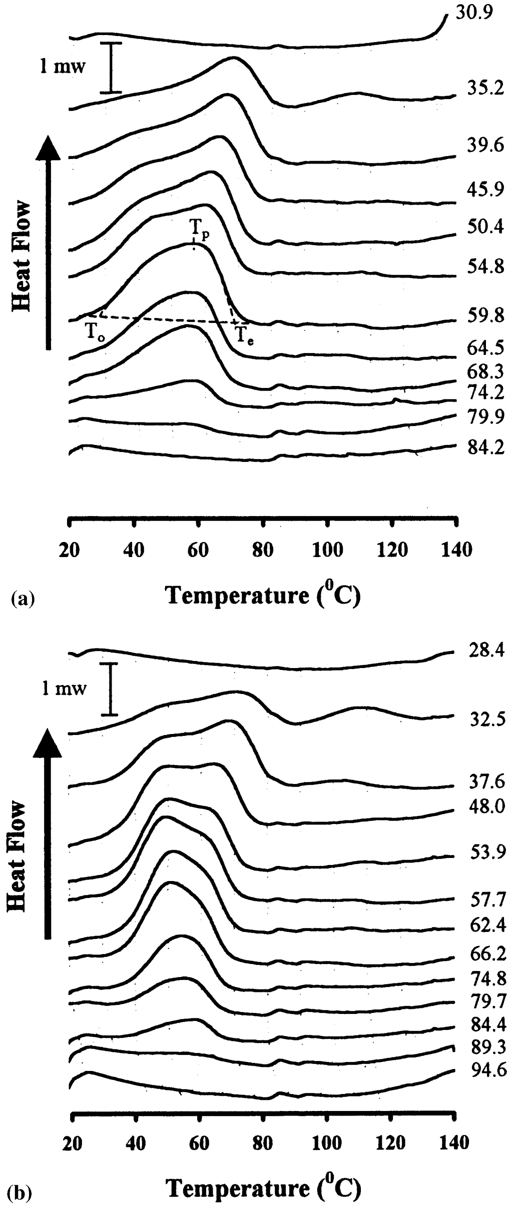


Fig. 3. DSC thermograms of *du wx* starch after initial heating to 140 °C and storage at 5 °C: (a) after 24 h and (b) after 7 days. Moisture content (%, w/w) is indicated for each thermogram. Heating rate was 10 °C/min (thermograms for moisture contents > 70% contained less starch, and thus the endotherm areas are not directly comparable as shown).

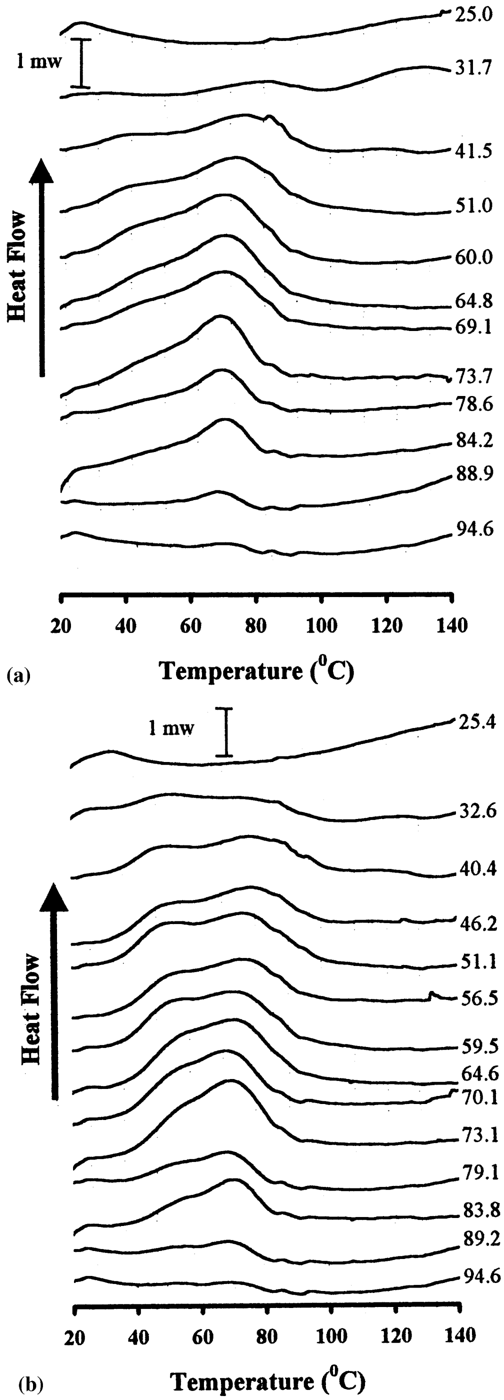


Fig. 4. DSC thermograms of *ae wx* starch after initial heating to 140 °C and storage at 5 °C: (a) after 24 h and (b) after 7 days. Moisture content (%, w/w) is indicated for each thermogram. Heating rate was 10 °C/min (thermograms for moisture contents > 70% contained less starch, and thus the endotherm areas are not directly comparable as shown).

Although the end temperature ($T_e$) of the retrogradation endotherm generally decreased as the moisture content increased (Fig. 5), the relationship between $T_e$ and moisture content was fundamentally different for *ae wx* starch than for the other three starches. The end temperature of *ae wx* starch is far higher than other starches, and it remained relatively unaffected by changes in moisture content in the region $<60\%$ moisture. After 1-day storage the end temperature of the retrogradation endotherm was similar to that of 7-day storage for all starches.

*Effect of moisture content and gelatinization heat treatment on retrogradation enthalpy.*— The effect of initial heating temperature on retrogradation enthalpy of the *wx*-containing maize starches and common corn starch at three different moisture contents is shown in Fig. 6. The effect of moisture content and initial heating temperatures on DSC thermograms for *du wx* starch and *ae wx* starch is shown in Figs. 7 and 8. Retrogradation endotherm shapes for common corn starch, *wx* starch and *su2 wx* starch were similar to that of *du wx* starch (Fig. 7), except that an endotherm for the amylose–lipid complex was also observed for common corn starch.

After 24-h storage, retrogradation enthalpy generally was highest at 50% moisture content and next highest at 65% moisture content. No retrogradation enthalpy was observed for *wx* starch with 80% moisture content after initial heating to 140 and 180 °C or with 65% moisture content after initial heating to 180 °C, or for *du wx* starch with 80% moisture content after initial heating to 180 °C. Retrogradation enthalpy was observed for all treatments for *ae wx* starch and common corn starch. Retrogradation enthalpy for *su2 wx* starch was

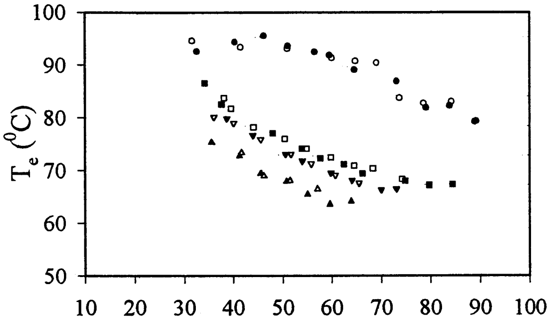
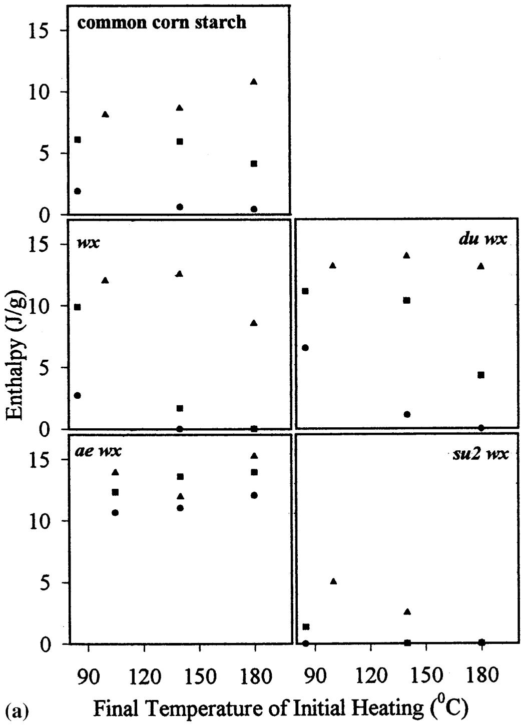


Fig. 5. End temperature of retrogradation endotherm of starches as a function of moisture content after storage at 5 °C for 24 h for *wx* starch (▽), *du wx* starch (□), *ae wx* starch (○), *su2 wx* starch (△). Solid symbols indicate storage of the same starches for 7 days.



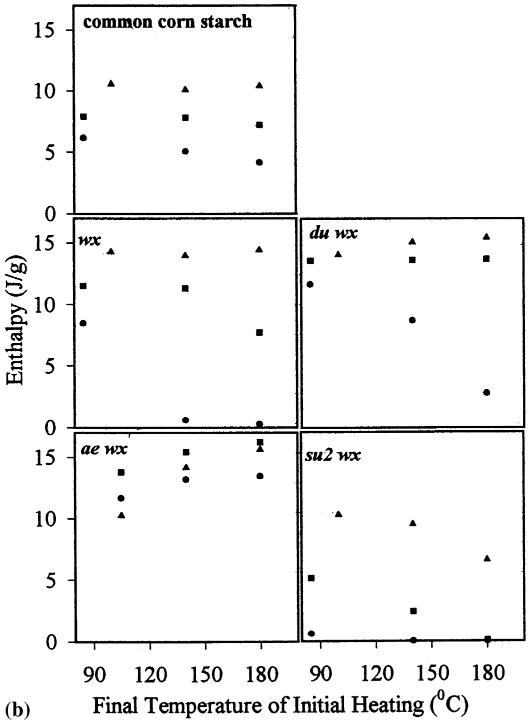


Fig. 6. The effect of initial heating temperature on starch retrogradation enthalpy at moisture content of ~80% (●), ~65% (■) and ~50% (▲): (a) after 24 h at 5 °C; (b) after 7 days at 5 °C.

far lower than for other starches when it was observed at all (Fig. 6(a)).

After 7-day storage, retrogradation enthalpy for *wx* starch at 50 and 65% moisture was greater than after 24-h storage, with the greatest increases for the 65% moisture sample previously heated to 140 or 180 °C. For *du wx* starch, the increase was greatest at 65% moisture previously heated to 180 °C. For *su2 wx* starch, an increase was observed for samples with both 50 and 65% moisture, but at 80% moisture very little retrogradation enthalpy was observed even for the lowest initial heating temperature. The behavior of the *ae wx* starch was distinct from the other three *wx*-type starches in that relatively little additional retrogradation enthalpy developed between days 1 and 7.

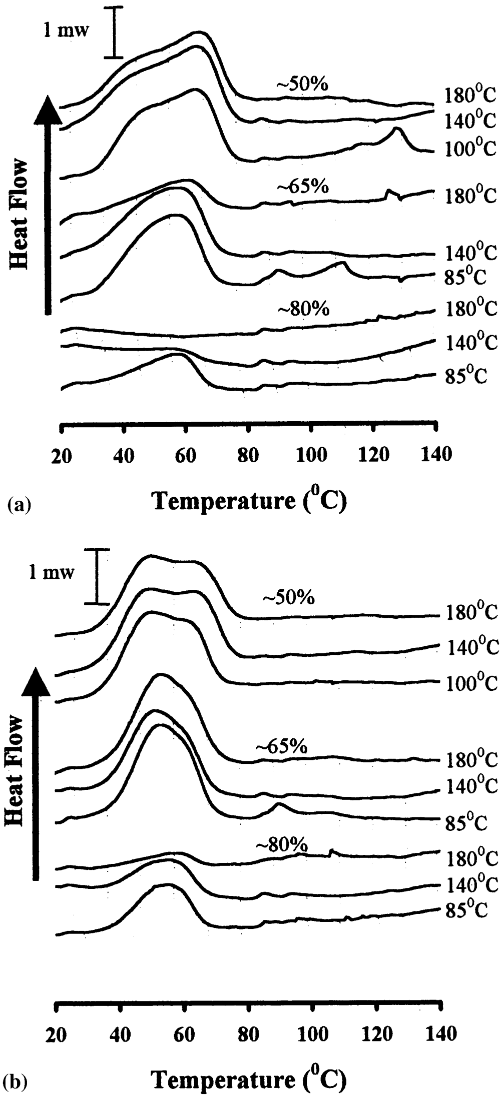


Fig. 7. DSC thermograms of *du wx* starch after different initial heating temperatures and storage at 5 °C: (a) after 24 h and (b) after 7 days. Moisture content (%, w/w) and initial heating temperature are indicated. Heating rate was 10 °C/min (thermograms for moisture contents > 70% contained less starch, and thus the endotherm areas are not directly comparable as shown).

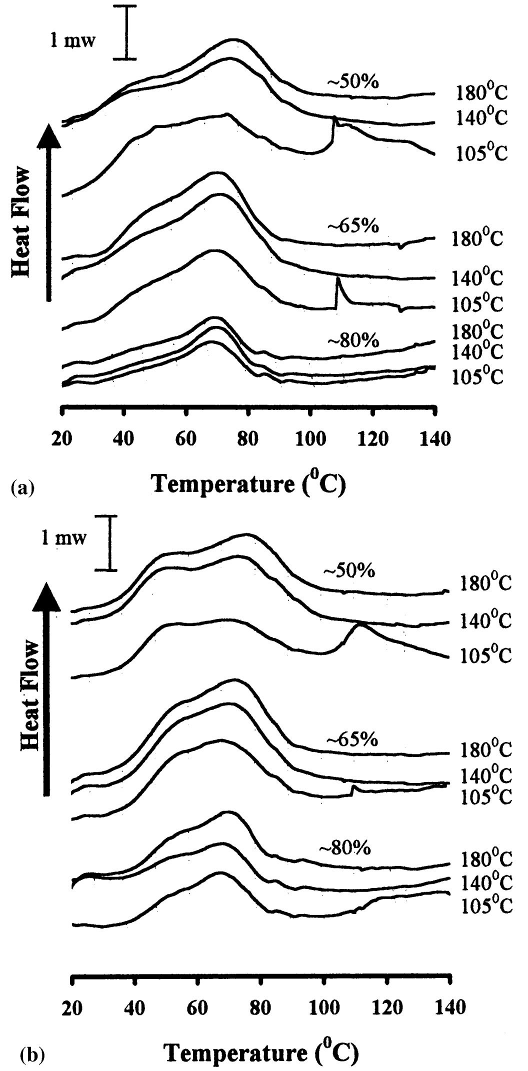


Fig. 8. DSC thermograms of *ae wx* starch after different initial heating temperatures and storage at 5 °C: (a) after 24 h and (b) after 7 days. Moisture content (%, w/w) and initial heating temperature are indicated. Heating rate was 10 °C/min (thermograms for moisture contents > 70% contained less starch, and thus the endotherm areas are not directly comparable as shown).

Common corn starch samples differed from the samples containing the *wx* genotype. Unlike for the *wx*, *du wx*, and *su2 wx* starches, there was not a profound effect of initial heating temperature, either at 1 or 7 days. Unlike for the *ae wx* starch, there was an appreciable increase in retrogradation enthalpy from day 1 to day 7 for the 80% moisture samples.

The nature of the DSC retrogradation thermograms varied with moisture content, initial heating temperature, and storage time. The thermogram shapes were generally similar for *wx*, *du wx*, and *su2 wx* starches, but these were distinct from the thermograms of *ae wx* starch.

The behavior of *du wx* starch is illustrated in Fig. 7. At 80% moisture, appreciable enthalpy was only observed at the lowest heating temperature after 24 h, with a peak temperature just below 60 °C. At 65% moisture, a lower-temperature shoulder was observed, particularly for the two lower initial heating temperatures. At 50% moisture, the low-temperature shoulder was pronounced for all initial heating temperatures. After 7-day storage, the magnitude of the lower-temperature shoulder increased for the 65 and 50% moisture samples. For the 50% moisture content, the magnitude of this peak was greatest for the lowest initial heating temperature. For the 65% moisture sample, the lower-temperature region dominated the thermogram in a way unrelated to initial heating temperature. The sample at 80% moisture showed little sign of more than one element in the peak for the lower heating temperatures; however, it did appear that the event for the sample heated to 180 °C and stored 7 days occurred at a higher temperature than for the other 80% moisture samples.

The behavior of *ae wx* starch after 24-h storage differed from that of the *du wx* starch in that an endotherm was observed for all treatments (Fig. 8). Little difference due to initial heating was observed at 80% moisture. As with *du wx* starch, at 50% moisture the importance of the low-temperature shoulder was greatest for the lowest initial heating temperature. After 7-day storage, the importance of the lower-temperature shoulder was greater for the lower initial heating temperatures, regardless of moisture content.

Thermograms for *ae wx* starch at 50% moisture also included a unique endotherm event just above 105 °C, but only for the samples heated to 105 °C. This event was absent in the samples heated to 140 °C or above.

*High-performance size-exclusion chromatograms for debranched* wx*-containing starch.*—Size-exclusion chromatograms for the isoamylase-debranched *wx*-containing starches are shown in Fig. 9. The debranched *wx* starch shows the characteristic chain length profile observed elsewhere [19,20]. The chromatogram for debranched *du wx* starch resembles that for *wx*, except that the short-chain peak (chains of DP $< 30$) is enhanced, as has been observed [19–21]. The *ae wx* starch is also characteristic of previously characterized *ae wx* starches from inbred lines [19,20] in that the long-chain peak (chains of DP $> 30$) is enhanced relative to *wx* starch, and the shape of short-chain peak is altered to favor a higher proportion of longer chains. The chromatogram for debranched *su2 wx* starch differs from that for *wx* starch in that the short chain peak may be considered to have two nearly equal components, and the shorter-chain component contains a higher proportion of area corresponding to unusually short chain lengths. For the differential refractive index chromatogram, the proportion of the total area for DP $\leq 12$ was 14% for *wx* starch, 14% for *du wx* starch, 8% for *ae wx*

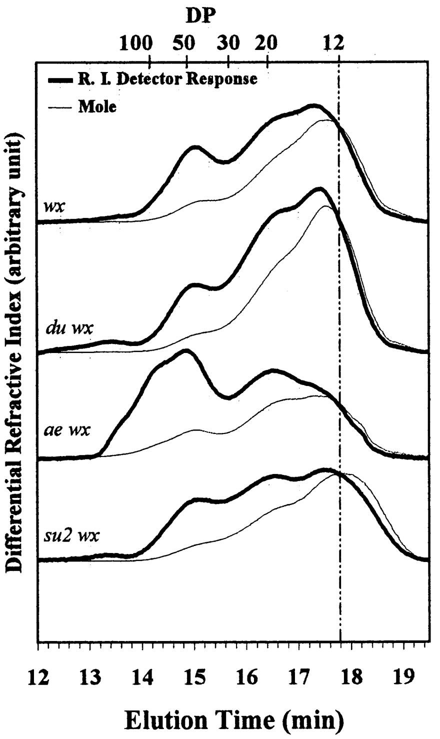


Fig. 9. High-performance size-exclusion chromatograms of debranched *wx*, *du wx*, *ae wx* and *su2 wx* starches.

starch, and 20% for *su2 wx* starch. The molar contribution of these short chains is a more substantial fraction of the total moles, as shown in Fig. 9.

## 4. Discussion

*Retrogradation as a function of moisture content.*—The enthalpy of gelatinization and melting combined remained approximately constant for all starches at moisture content $>50\%$ (Fig. 1). The enthalpy of *ae wx* starch was clearly the highest, while that of *su2 wx* was lowest, consistent with prior observations [19,20,25].

Retrogradation enthalpy as a function of moisture content showed the same general pattern for each starch: a maximum at some intermediate moisture content (Fig. 2), consistent with previous studies [7,8]. That the starches were all of the *wx*-type indicates that the amylopectin is responsible for the different response to moisture content.

The failure to observe retrogradation as moisture content approaches 30% is undoubtedly related to the previously observed low enthalpy of gelatinization plus melting at these low moisture levels [11]. Working with common corn starch, Jouppila and Roos [26] observed limited retrogradation at moisture contents as low as 20%, but only after holding at 50–60 °C. They suggested that these higher storage temperatures were necessary to make $T_{\text{storage}} - T_{\text{g}}$ sufficiently large for sufficient polymer mobility.

Comparison of enthalpy after 24 h and 7 days for each starch shows little or no change in the low-moisture region (Fig. 2(a,b)). The magnitude of the increase in the maximum enthalpy value at intermediate moisture content during this period was in the order *su2 wx* > *wx* > *du wx* > *ae wx*. Previous work has shown that retrogradation rate varies considerately among these starches [5,23,25]. Since a limiting value is reached quickly for the *ae wx* starch, little change can occur after 24 h. Retrogradation of *ae wx* starch is even more rapid than for the potato amylopectin described by Kalichevsky et al. [27] and Wursch and Gumy [28].

The four starches also differed considerably in the dependence of retrogradation enthalpy on moisture content (Fig. 2). The *ae wx* starch showed measureable retrogradation at 95% moisture after only 24-h storage, whereas no retrogradation after 24 h was observed for *su2 wx* starch above 60% moisture. In the high-moisture region, the pattern of the relationship between the retrogradation enthalpy and the moisture content was clearly different for the four starches after 24 h and after 7 days.

Previous studies have shown that for 70% moisture, retrogradation rate is a function of the initial heating temperature for gelatinization [5,23] up to 180 °C. Loss of residual order was proposed to explain this observation. At this moisture content, granules could swell no more than two to three times in volume. Amylopectin molecules would be expected to contain no residual double helices after heating to the higher temperatures; however, the general orientation of the two chains which had been in the same double helix might be increasingly lost as the initial heating temperature during gelatinization increases. At 70% moisture content, the different tendency toward retrogradation for the four starches would reflect the different general propensity of the chains to associate and any difference in the extent of loss of residual order; both would be governed by the molecular structure of the amylopectin.

Yuan et al. [19] and Thompson and Blanshard [29] have studied retrogradation of *wx*, *du wx*, and *ae wx* starches at about 70 and 90% moisture content, and have observed a concentration dependence in the order *wx* > *du wx* > *ae wx*, consistent with the data in the Fig. 2.

Retrogradation endotherms often had a low-temperature and a high-temperature component (Figs. 3 and 4), as previously described [25,28]. For *du wx* starch stored for 24 h, the high-temperature component was observed at the highest moisture content, both components were observed for intermediate moisture contents and the higher-temperature component was pronounced at the lower moisture contents (Fig. 3). For waxy starch, Wursch and Gumy [28] stated that only the low-temperature component was present at high dilution, but no endotherm was presented.

However, only when a range of moisture contents is compared, as in the present report (Fig. 3), is it clear how the two components may be understood; if the 74.8% moisture sample stored 7 days were considered alone, it might appear to be only the low-temperature component. After 7-day storage the low-temperature region of the endotherm becomes more prominent, especially at the intermediate moisture level, consistent with previous observations [25,28]. Close inspection of 24-h and 7-day thermograms for similar intermediate moisture contents reveals that the high-temperature region has remained similar, while the low-temperature region has shifted such that a nearly equal area has moved from the region just below about 40 °C to the region just above 40 °C. Others have characterized the thermograms as undergoing a slight increase in $T_o$ [4,25]. The increase in total enthalpy (see Fig. 2(a,b)) is slight. At the higher moisture contents, increased enthalpy between 24 h and 7 days is observed over the entire range of the endotherm.

Wursch and Gumy [28] suggested that the two components might result from melting, retrogradation (reordering), and a second melting, all during the heating during thermal analysis. This behavior seems unlikely since the retrogradation kinetics would have to be exceptionally rapid. Although polymer recrystallization after only partial melting is well described [30], we suggest that this possibility is inconsistent with the general increase in the low-temperature component on storage.

At intermediate moisture content for *du wx* starch, two components are readily apparent, and the low-temperature component develops between 24 h and 7 days. Assuming the observed enthalpy is due to dissociation of double helices [31], then two populations of double helices may form. These populations could result from chain association of the full lengths of exterior chains of amylopectin, or they could result from chain association involving only a portion of the exterior chains due to constraints on double helix formation. We suggest that the high-temperature peak results from long-chain double helices that form rapidly. At intermediate moisture content, the low-temperature region results from shorter double helices, due to either shorter chains or sub-optimal chain association. The shortest of these shorter double helices would be the least stable, and over 7 days the component chains could re-orient for more efficient double helix formation, accounting for the shift of the area of the low temperature shoulder (just below 40 °C) to the low temperature peak (just above 40 °C). Our interpretation is similar to the suggestion made by Ward et al. [4] that crystallites of amylopectin annealed during storage. The amount of starch as double helix might remain constant, explaining the near-constant total enthalpy. We envision this process of partial annealing during storage to occur within a framework fixed by the initially formed double helices. Alternatively, annealing might occur in the absence of a rigid framework, as Waigh et al. [32] have suggested that, even though double helices may be considered as rigid structural elements, these elements themselves may be mobile. In either case, long double helices would serve as a constraint on mobility of remaining chains.

That the lower-temperature component is not as pronounced for *du wx* starch at higher moisture, and that enthalpy develops more slowly at higher moisture [29] may be related. The slower double helix formation may allow initial helices to organize more efficiently, leading to fewer constraints on subsequent double helix formation. Fewer constraints should allow more of the chains to participate in double helices to the fullest extent.

The retrogradation endotherms for *ae wx* starch as a function of moisture content show a generally similar pattern. After 24-h storage two components are apparent, especially at intermediate moisture contents. By 7 days the low-temperature component is more pronounced, but as for *du wx* starch the difference is largely due to a shift of enthalpy from the region below 40 °C to the region above it. The two-component nature may be more readily apparent than for *du wx* starch since the high-temperature component is at higher temperature for the *ae wx* starch. The endotherm shapes for *ae wx* starch differ from those of *du wx* starch at the higher moisture contents after 7 days, in that the two components are readily apparent for *ae wx* starch even at the highest moisture content. This observation may be related to the loss of the long lead shoulder observed in thermograms for *ae wx* starch after 24 h. Extremely rapid formation of the longer-chain double helices could provide more extensive initial constraints on formation of subsequent double helices, leading to greater annealing over time within the structure established by the longer double helices.

We suggest that the different patterns of $T_e$ as a function of moisture content (Fig. 5) may be related to formation of different length double helices. That $T_e$ generally increases as moisture content decreases may be related to the idea that solvent-assisted helix dissociation is less readily accomplished as solvent availability decreases. Lower polymer mobility due to smaller $T - T_g$ [26] might also explain this observation.

Eerlingen et al. [33] showed that a two-component retrogradation endotherm resulted for waxy maize at 50% moisture stored for 2 days at room temperature; however, when the starch was stored for 1 day at 6 °C and then 2 days at 40 °C, only an endotherm in the higher temperature region was observed. After another 27 days at 40 °C, the peak maximum was even higher, at ~70 °C. Consequently it appears that storage at 4 °C in the present report may have effectively nucleated the retrogradation process but hindered the perfection of the double helices and perhaps of the crystallites that resulted.

*Retrogradation as a function of both moisture content and initial heating temperature.*— Fig. 6 shows the combined influence of moisture content and initial heating temperature on retrogradation of the four starches and a common corn starch. The *wx* starch at 65 and 80% moisture content was profoundly affected by initial heating temperature, consistent with previous work [5] in which retrogradation of *wx* starch at 70% moisture was evaluated after heating to a wide range of initial heating temperatures. However, at 50% moisture, the effect of initial heating temperature was much diminished. The *du wx* starch exhibited the same general behavior, even though the effect of initial heating temperature was not as great. Previous work [5] showed relatively little effect of initial heating temperature on retrogradation of *ae wx* starch, and Fig. 6 is consistent with those observations. Although it is difficult to evaluate the effect of initial heating temperature on *su2 wx* starch due to the limited retrogradation observed, the effect is best observed after 7-day storage. A strong influence of concentration is evident as well after 7 days.

Although retrogradation enthalpy is affected by both moisture content and initial heating temperature for *wx*, *du wx*, and *su2 wx* starches, it is little affected by either moisture content or initial heating temperature for *ae wx* starch. The explanation for this behavior may be related to the length of double helices in the four starches. Either heating to a higher temperature or a higher moisture content could result in more fully disordered amylopectin, accounting for the behavior of the *wx*, *du wx*, and *su2 wx* starches. Eliasson and Gudmundsson [34] have pointed out that for temperatures < 90 °C and concentrations of 8–12% (w/w) starch, similar rheological properties could result from manipulation of either heating regime or concentration. The present report suggests that this thought may have application over a much wider range of conditions. The longer chains of the *ae wx* starch may contribute to much-enhanced retrogradation kinetics, thus allowing nearly complete retrogradation on a time scale too short to observe any effects of initial heating temperature or concentration, even after extensive chain disordering.

Retrogradation of the common corn starch showed a relatively strong concentration dependence, but little or no effect of initial heating temperature. Retrogradation enthalpy was lower than for the *wx* starch at 50% moisture content after 7 days, an observation that may be understood as the result of the lower amylopectin content, as the literature would suggest that retrogradation below 100 °C by DSC is primarily due to the amylopectin [35].

Since the amylopectin of common corn starch is thought to resemble that of *wx* starch [36], it would appear that the amylose of common corn starch may be somehow related to the lack of an effect of initial heating temperature. Klucinec and Thompson (accepted) have shown that amylose likely interacts with amylopectin during retrogradation of common corn starch and high-amylose starches.

After 180 °C heating of *du wx* starch and 24-h storage, no endotherm was observed for 80% moisture content, a single high-temperature endotherm was observed for 65% moisture content, and a two-component endotherm was observed for 50% moisture content. After 7-day storage the lower-temperature region became dominant at 65 and 50% moisture content, whereas after 7 days only a high-temperature peak was apparent at 80% moisture content. This latter observation may be related to the work of Liu and Thompson [23], in which initial retrogradation of *du wx* starch heated to 180 °C was first observed in the high-temperature region for 70% moisture content. That work also suggested that when the initial heating temperature was $< 140$ °C, two rate constants best described retrogradation of *du wx* starch. The authors suggested that these rate constants might describe two different processes related to the two regions of the retrogradation endotherm [23]. The processes may involve formation of two populations of double helices: more rapidly forming longer double helices, and shorter double helices where formation is constrained by the longer helices.

Comparison of the retrogradation thermograms might help explain why initial heating temperature and moisture content (in the range 50–80%) was observed to be important for retrogradation of *du wx* starch but not for *ae wx* starch. After 24-h storage of *ae wx* starch, the major element of the endotherm is the high-temperature component, regardless of moisture content or initial heating temperature. We suggest that for *ae wx* starch the process is driven by the longer chains rapidly forming double helices at all temperature and moisture content combinations. Although the low-temperature region becomes somewhat more prominent after 7 days, annealing of chains in the shortest double helices, with minimal increase in total double helical content, could account for the change. This suggestion notwithstanding, at moisture contents above 80%, even the *ae wx* starch showed decreased retrogradation enthalpy.

*Amylopectin structure of the four starches.*—The chromatograms showing the distribution of chain lengths for each starch are characteristic for each starch (Fig. 9). Those for *wx*, *du wx*, and *ae wx* starches agree well with previous work with these genotypes from inbred lines [19,20]. A chromatogram for debranched *su2 wx* starch is not available in the literature, although Fuwa et al. [37] reported that debranched *su2 wx* starch had a high proportion of what they termed ‘fraction III’, corresponding to what we have called the short-chain peak (by their analysis *su2 wx* starch had a lower proportion in this peak than did debranched *du wx* starch [37]). Our work shows that *su2 wx* starch has the highest proportion of its area as DP 12 or below (Fig. 9). The DP of the peak maximum is also slightly lower than for *wx* or *du wx* starches. On a molar basis, the proportion of these shortest chains is very high. Shi and Seib [38] suggested that even the small variation they observed in the proportion of chains with DP 6–9 could account for difference in retrogradation in single mutant *wx* starches from different sources.

Fig. 10 shows plausible models for two adjacent clusters for each starch. The clusters for *du wx* starch have a larger number of chains than for *wx* starch. This model takes into account the fewer long B chains for *du wx* starch [19] and the similar chain length maxima for the two starches. The model for *ae wx* starch has a small number of chains in a cluster, a feature derived from the observed high proportion of long B chains [19], and longer helices due to the relatively long population of A and B1 chains, which account for the bulk of the short chain peak [19]. This model for *ae wx* starch is consistent with a cluster model proposed by Jane et al. [39] for potato amylopectin and *ae wx* starch; however, the retrogradation rate for the *ae wx* starch is greater than that reported for potato amylopectin [28]; consequently, the same model may not apply to both. The model for *su2 wx* starch has a large number of very short chains which would either inhibit double helix formation (not shown in Fig. 10) or form

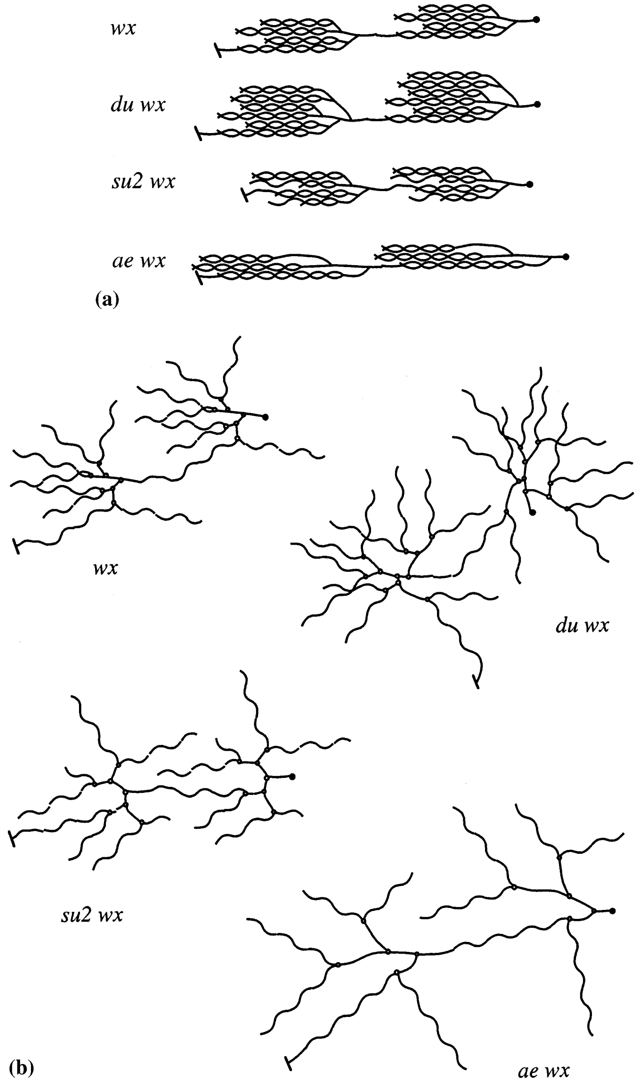


Fig. 10. Models for clusters for *wx*, *du wx*, *su2 wx*, and *ae wx* starches. (a) Two adjacent clusters as they might occur in a native starch granule. The lines indicate chains of 1,4-linked anhydroglucose glucose units (AGUs). Double helices are indicated, with one complete turn of a chain (six AGUs) corresponding to two helix repeat units. Chain DPs are proportional to the length of the lines. The reducing end is indicated by the filled circle. Possible extension of a non-reducing terminus to the next adjacent cluster is indicated by a slash. (b) Two disordered clusters after gelatinization, illustrating the non-random distribution of branch points, as described by Galinsky and Burchard [41,42]. Chain lengths correspond to those in (a). The branch points are emphasized, and indicated as open circles. The way more than two clusters are joined is not clear, as they may be joined linearly or in a manner in which higher-order branching results. The extent of disordering may be related to moisture content and the initial heating condition. Less disordered chains might remain in an orientation more similar to the original orientation in the double helix.

poorly stable double helices (illustrated in Fig. 10 as the shortest double helices, representing six glucose units in each chain). Gidley and Bulpin [40] showed that only chains of DP $\geq 6$ could participate in a double helix when associated with a longer chain. Unusually unstable double helices in the native starch would explain the unusual behavior observed by Liu and Thompson [23], in which annealing was observed well below the gelatinization onset temperature. After loss of the ordered structure on gelatinization, chains too short to form stable double helices would serve to dilute the longer chains and sterically interfere with formation of double helices from longer chains, thus retarding the retrogradation process.

*The present work in perspective.*—Even 80% moisture content and 20% starch represents a system in which granule swelling would be constrained to about three times the initial granule diameter. The 90–95% moisture samples would allow a 10–20-fold volume increase, close to maximal swelling for most granules. A standard moisture content in the Brabender Viscoamylograph is 92% (w/w).

The current work has not investigated development of crystallinity during retrogradation; however, Thompson and Blanshard [29] showed that by wide-angle X-ray diffraction, crystallinity developed rapidly for *ae wx* starch at 27% solids and only somewhat less slowly for *ae wx* starch at 10% solids. Crystallinity increases also paralleled the slower enthalpy development for *wx* and *du wx* starches in that work. In that work not only did double helices form, but they associated in at least semi-crystalline register [29]. The rate at which retrogradation enthalpy development occurred is consistent with enthalpy development in the present report. Cook and Gidley [31] have suggested that DSC monitors double helix dissociation, and that energy associated with loss of crystalline packing is negligible. That the three of these starches previously studied [29] all readily increase in crystallinity suggests that not only do the double helices form, but there is sufficient flexibility of the interior portion of the B chains that alignment and organization of double helices may occur as well.

For some time it has been known that both *du wx* and *ae wx* starch retrograde more rapidly than *wx* starch [19,20]. Since *ae wx* starch has longer chains overall, and *du wx* starch has a shorter average chain length, it has been challenging to explain retrogradation behavior based on amylopectin fine structure. We suggest that formation of the longest double helices drives the process in both starches, and the exceptionally long chain lengths of *ae wx* starch are responsible for its exceptionally rapid retrogradation. We suggest that double helix formation in retrogradation will also be favored for molecules in which the orientation of chains is similar to their initial double-helical orientation, i.e., in which chain disordering is minimal. Galinsky and Burchard [41,42] have described amylopectin as a non-randomly branched co-polymer, and emphasized that the periodicity in branching is an important structural feature in this type of disordered polymer. Fig. 10(b) illustrates this concept for disordered clusters of each starch, showing how the relationship among branch points might influence chain mobility. It is evident that clusters which contain a large number of branch points in close proximity will also be those with the greatest steric constraint on large-scale motion of the external chains. Since the *du wx* starch has the most highly branched clusters, this starch would have the greatest constraint on large-scale chain freedom after loss of double-helical structure. Thus greater polymer memory is proposed to account for the more rapid retrogradation of *du wx* starch compared to *wx* starch. Extensive chain clustering would also explain why *du wx* starch is less sensitive to moisture content than is *wx* starch, since the chains in each cluster of *du wx* starch are structurally constrained to a limited volume by the branch pattern. These constraints might also explain the reported more limiting swelling power of *du wx* starch [21,43]. Although the mobility of the external chains of *ae wx* starch is far less constrained by the branching arrangement, the greater length of these chains might account for a strong drive toward double helix formation, sufficient to rapidly overcome extensive disordering resulting from high dilution or heating to high temperatures.

Aberle and Burchard [44] have suggested that, as moisture content decreases, amylopectin molecules would necessarily diminish in volume because of the constraints on their ability to interpenetrate. This behavior would bring external chains in greater proximity and enhance double helix formation. We propose that this mechanism is related to the general increase in retrogradation enthalpy as moisture content decreases from the high-moisture to the intermediate-moisture range. We suggest that analogous reasoning could be applied to individual clusters within the amylopectin molecules. Given the proposed difference in cluster structure, differences in the manner in which a decrease in moisture influences the decreased cluster volume might be anticipated. In this way variation in the cluster structure could help explain the different effects of moisture content on retrogradation for these four starches.

## 5. Conclusion

The four *wx*-type starches studied here differ in the way retrogradation behavior is a function of moisture content. Previous work has shown that retrogradation of these four starches varies with initial heating temperature in gelatinization. Retrogradation of the *wx*, *du wx*, and *su2 wx* starches is strongly influenced by moisture content (in the range 50–80%) and initial heating temperature, whereas retrogradation of *ae wx* starch is minimally influenced by either. The difference in retrogradation behaviors is likely related to the different amylopectin structures. Models are presented to explain the different retrogradation behaviors.

## Acknowledgements

We thank Jeff Klucinec for assistance with the high-performance size-exclusion chromatography.

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