

Retrogradation of *du wx* and *su2 wx* Maize Starches After Different Gelatinization Heat Treatments

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ABSTRACT

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Retrogradation of *du wx* and *su2 wx* starches after different gelatinization heat treatments was studied by differential scanning calorimetry. Suspensions of 30% (w/w) starch were initially heated to final temperatures of 55–180°C. Gelatinized starch was cooled and stored at 4°C. Starch retrogradation in the storage period was influenced by initial heat treatments. Retrogradation of *du wx* starch was rapid: when initially heated to 80–105°C, retrogradation enthalpy was ≈10 J/g after one day at 4°C. The retrogradation enthalpy was ≈15 J/g after 22 days of storage, and reached a maximum of 16.2 J/g after 40 days of storage. For *du wx* starch, appli-

cation of the Avrami equation to increases in retrogradation enthalpy suggests retrogradation kinetics vary with initial heating temperature. Furthermore, starch retrogradation may not fit simple Avrami theory for initial heating ≤140°C. Retrogradation of *su2 wx* starch was slow. After 30 days of storage at 4°C, the maximum retrogradation enthalpy for all initial heating temperatures tested was 7.0 J/g, for the initial heating to 80°C. This work indicates that gelatinization heat treatment in these starches is an important factor in amylopectin retrogradation, and that the effect of initial heat treatment varies according to the genotype.

Starch retrogradation is the process that occurs when starch molecules reassociate and form an ordered structure during storage. In an initial step, two chains may associate. Ultimately, under favorable conditions, a crystalline order appears and physical phase separation occurs (Atwell et al 1988). Retrogradation is important in industrial use of starch as it can be a desired end point in certain applications, but it also causes instability in starch pastes.

Starch retrogradation is influenced by the botanical source (Orford et al 1987, Roulet et al 1990) and the fine structure of amylopectin (Kalichevsky et al 1990, Ward et al 1994). The amylose-to-amylopectin ratio (Russell 1987, Gudmundsson and Eliasson 1990) affects the kinetics of retrogradation. In nonmutant genotype starches, the amylose is responsible for short-term (less than one day) changes (Goodfellow and Wilson 1990). The amylopectin molecule is responsible for longer term rheological and structural changes of starch gels (Gudmundsson 1994). Water content in the starch gel (Zeleznek and Hoseney 1986, Keetels et al 1996) and storage temperature (Colwell et al 1969) can affect the rate and extent of starch retrogradation. Certain polar lipids and surfactants (Gudmundsson 1994) and other components (Biliaderis and Prokopowich 1994) can retard or reduce these changes.

To study starch retrogradation, several techniques have been used, including thermal analysis by differential scanning calorimetry (DSC) (Russell 1987, Slade and Levine 1991, Fisher and Thompson 1997), X-ray diffraction (Ring et al 1987, Liu 1997), rheological analysis (Ring et al 1987, Clark et al 1989, Roulet et al 1990, Keetels et al 1996, Yuan and Thompson 1998), Fourier transform infrared spectroscopy (Goodfellow and Wilson 1990, van Soest et al 1994, Liu 1997), Raman spectroscopy (Bulkin et al 1987), and microscopy (Jacobson et al 1997).

Retrogradation of starch has been studied using the Avrami equation. The Avrami equation has been used to model crystallization of starch gels (McIver et al 1968, Colwell et al 1969, Wong and Lelievre 1982, Longton and LeGrys 1981, Marsh and Blanshard 1988), starch retrogradation (Bulkin et al 1987; Inouchi et al 1991b), and bread staling (Fearn and Russell 1982, Russell 1983a–c). Some authors determined the crystallization rate constant (k) when the modes of nucleation and growth were assumed (Fearn and Russell 1982, Russell 1983a–c, van Soest et al 1994). Others used Avrami plots to determine the Avrami index (n) and gain

insight into the mechanism of crystallization (McIver et al 1968; Wong and Lelievre 1982; Inouchi et al 1991b). Kinetics of crystallization of starch depends on the storage temperature (Longton and LeGrys 1981, Marsh and Blanshard 1988) and starch concentration (Longton and LeGrys 1981). For starch, the mechanism of starch crystallization is still not well described, and the value of the Avrami index does not have unambiguous meaning. Consequently, the strategy of assuming a crystallization mechanism is inappropriate; determination of Avrami index should be done concurrently with determination of the rate constant of starch retrogradation. Recently, the kinetic processes in starch retrogradation have been addressed by quantification of the extent of the band narrowing and of the time-dependent intensity changes of specific bands by Raman spectroscopy (Bulkin et al 1987) and infrared spectroscopy (Goodfellow and Wilson 1990).

Fisher and Thompson (1997) demonstrated that the temperature of initial heat treatment, even above the DSC gelatinization completion temperature, affects the retrogradation rate of *wx* and *ae wx* maize starch. The purpose of the present study was to follow up on the work of Fisher and Thompson (1997) by investigating the effect of initial heating temperature on retrogradation of *du wx* and *su2 wx* starches, and to expand on the previous approach by applying the Avrami equation to gain insight into the differences in retrogradation rate of *du wx* starch after initial heating to temperatures of 80–180°C.

MATERIALS AND METHODS

The *du wx* starch was obtained from American Maize Products Company (now Cerestar USA, Hammond, IN). The *su2 wx* starch was obtained from National Starch and Chemical Co. (Bridgewater, NJ). Moisture content, measured by Approved Method 44-15A (AACC 1995), was 10.3% for *du wx* starch and 11.3% for *su2 wx* starch.

Heat treatment of the starch suspensions and the thermal analysis of retrogradation were done using a differential scanning calorimeter (DSC-7, Perkin-Elmer Corp., Norwalk, CT) equipped with a thermal analysis data station. Starch was weighed accurately into stainless steel sample pans (319-1605, Perkin-Elmer). Deionized water was added to make suspensions of ≈30% starch solids. Total mass of the pan contents was ≈50–60 mg. Pans were sealed and equilibrated for 12–24 hr at room temperature. Calibration was done with indium. An empty pan was used as a reference.

The gelatinization temperature range was determined from the endotherm during the heating of a 30% starch suspension to 140°C at 10°C/min. Some of the 30% starch suspensions were heated to temperatures (referred to as initial heating temperatures) below

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the gelatinization endotherm completion temperature (T_c), and were either held at that temperature for 20 min or not. All samples were then quench-cooled to 5°C. After 2 min (to stabilize the calorimeter), samples were reheated from 5 to 140°C at 10°C/min. Onset temperature (T_o), peak temperature (T_p), and enthalpy (ΔH) for residual gelatinization were obtained from the reheating endotherm.

Other samples were initially heated at a rate of 10°C/min from 5°C to various temperatures up to 180°C and cooled immediately at

a rate of 10°C/min to 5°C. Samples were then immediately removed from the calorimeter and stored in a refrigerator (4°C). After the appropriate storage time, the sample pan was removed from the refrigerator and immediately put into the sample holder of the calorimeter. Independent samples for each initial heating temperature were prepared for each storage time. Stored samples were heated from 5 to 140°C at 10°C/min.

For *du wx* starch, the data were analyzed by fitting the Avrami equation:

$$\Phi = (\Delta H_L - \Delta H_t) / (\Delta H_L - \Delta H_0) = \exp(-kt^n)$$

where Φ (the fraction amorphous) (Hiemenz 1984) is the fraction of the total change in retrogradation endotherm enthalpy still to occur; ΔH_L , ΔH_t , and ΔH_0 are the enthalpy (on a dry starch basis) at infinity (complete retrogradation), t time, and zero time, respec-

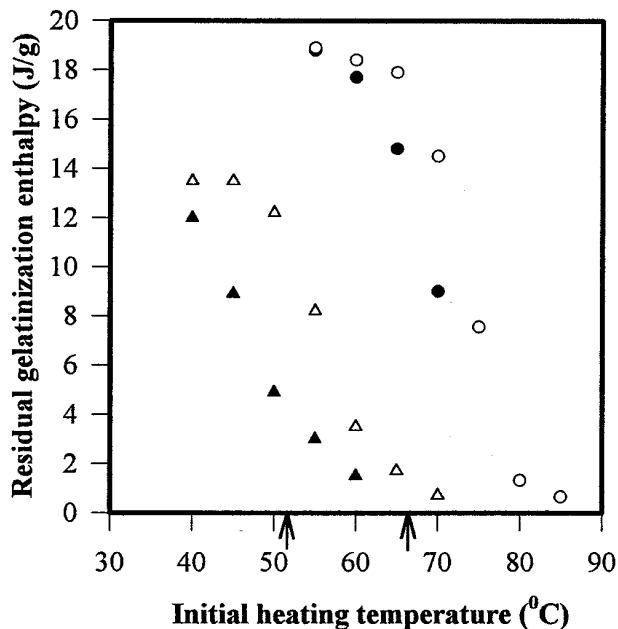


Fig. 1. Effect of initial heating temperature within the gelatinization range for 30% *du wx* starch on residual gelatinization immediately after heating (○) and immediately after heating and 20 min of holding (●). Effect of initial heating temperature for 30% *su2 wx* starch on residual gelatinization immediately after heating (△) and immediately after heating and 20 min of holding (▲). Arrows indicate the onset temperatures for *su2 wx* (51.9°C) and *du wx* (66.1°C) starches.

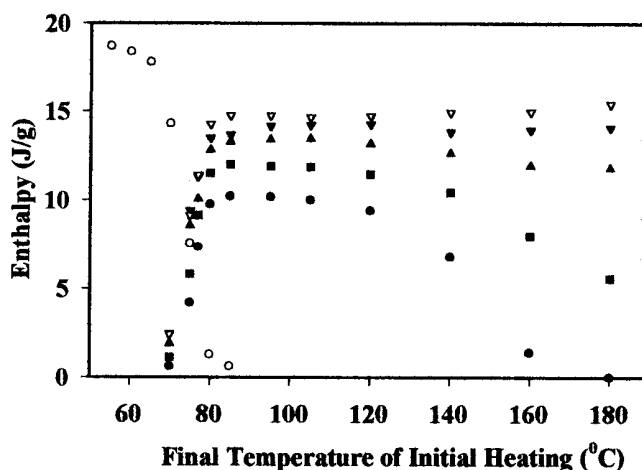


Fig. 2. Retrogradation of 30% *du wx* gelatinized starch as a function of initial heating temperature. Residual gelatinization enthalpy immediately after initial heating (○). Retrogradation enthalpy measured after one day (●), three days (■), seven days (▲), 12 days (▼), and 22 days (▽).

TABLE I
Residual Gelatinization Endotherms^a of *du wx* and *su2 wx* Starch at 30% Solids Content After Different Thermal Treatments^b

Thermal Treatment Initial Heating Temperature (°C)	<i>du wx</i>			<i>su2 wx</i>		
	T_o (°C)	T_p (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	ΔH (J/g)
Control ^c	66.1 ± 0.2	77.0 ± 0.3	18.4 ± 0.1	51.9 ± 0.6	58.2 ± 0.3	12.5 ± 0.2
With 20 min of holding						
40	nd ^d	nd	nd	52.6	59.5	12.0
45	nd	nd	nd	55.5	61.0	8.9
50	nd	nd	nd	60.7	65.9	4.9
55	68.5	78.0	18.8	65.9	70.5	3.0
60	71.2	78.5	17.7	70.1	74.5	1.5
65	75.3	80.4	14.8	nc ^e	nc	nc
70	79.4	82.8	9.0	nc	nc	nc
Without 20 min of holding						
40	nd	nd	nd	52.2	58.9	13.5
45	nd	nd	nd	52.7	58.9	13.5
50	nd	nd	nd	52.4	59.3	12.2
55	66.9	78.2	18.9	54.7	61.5	8.2
60	67.2	78.0	18.4	60.9	67.4	3.5
65	68.4	78.0	17.9	66.3	72.7	1.7
70	72.5	78.8	14.5	71.8	76.3	0.7
75	77.0	81.5	7.6	nc	nc	nc
80	81.7	85.2	1.3	nd	nd	nd
85	89.0	94.2	0.65	nd	nd	nd

^a Onset temperature (T_o), peak temperature (T_p), and enthalpy (ΔH) for residual gelatinization were obtained from the reheating endotherm.

^b Starch suspensions were heated to temperatures below the gelatinization endotherm completion temperature ($T_c = 87.4 \pm 0.6^\circ\text{C}$ for *du wx* starch, $80 \pm 0.9^\circ\text{C}$ for *su2 wx* starch) and were either held at that temperature for 20 min or not.

^c Results from triplicate samples reported as mean ± standard deviation.

^d Not determined; no data were collected for these samples.

^e Not calculated because residual gelatinization endotherm was not observed.

tively; k is the rate constant; and n is the Avrami exponent. For this calculation, ΔH_L was considered to be ΔH on or after 40 days. The ΔH_0 was defined as zero since no retrogradation endotherm was observed on immediate reheating for *du wx* starch. Data were subjected to linear regression (least square) analysis to determine best-fit values of k and n . The correlation coefficient (r^2) of this fit is also obtained. To apply the Avrami theory, we assumed that retrogradation enthalpy was linearly related to crystallinity.

RESULTS

Effect of Initial Heat Treatment Below T_c on Residual Gelatinization Enthalpy

The effect of initial heat treatment (initial heating temperature and holding time at that temperature) on residual gelatinization enthalpy is shown in Table I and Fig. 1. The T_o and T_p of the residual gelatinization endotherm increased when the initial heating temperature was below T_c and yet high enough to alter the endotherm. Heating to below T_o (51.9°C for *su2 wx* starch and 66.1°C for *du wx* starch) had different effects on residual gelatinization for *du wx* and *su2 wx* starches (Table I). Heating to approximately T_o and immediate cooling had very little effect on residual gelatinization enthalpy of either starch (Table I and Fig. 1). However, after 20 min holding at 50°C (1.9°C below T_o), the residual gelatinization enthalpy of *su2 wx* starch decreased by >8 J/g (Fig. 1). After holding 20 min at 65°C (1.1°C below the T_o), the residual gelatinization enthalpy of *du wx* starch decreased <4 J/g. It is evident that holding at 50°C (1.9°C below T_o) for 20 min before cooling the *su2 wx* starch led to a more extensive loss of gelatinization enthalpy than holding at 65°C (1.1°C below T_o) for 20 min before cooling the *du wx* starch (Fig. 1). After heating to temperatures within the gelatinization range without 20 min holding, residual gelatinization enthalpy decreased as the initial heating temperature increased. For *du wx* starch, residual gelatinization enthalpy was almost absent after heating the sample to 85°C (Fig. 1).

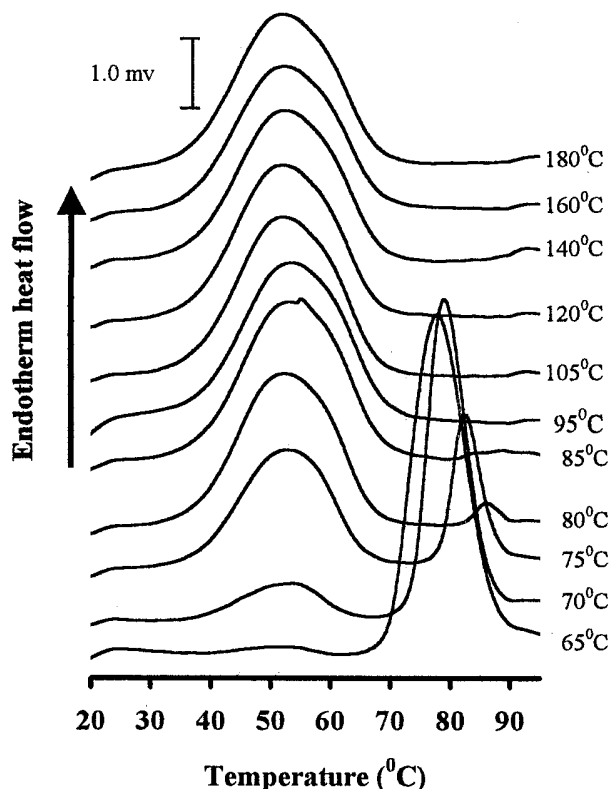


Fig. 3. Typical thermograms for retrogradation for 30% *du wx* starch after different initial heating temperatures and storage for 12 days at 4°C. Labeling refers to initial heating temperature.

Retrogradation of *du wx* and *su2 wx* Starches After Heating to Various Initial Heating Temperatures Above T_c

After one day of storage, a retrogradation endotherm for *du wx* starch was observed in all the DSC thermograms except for the initial heating temperature of 180°C (Fig. 2). The retrogradation endotherm peak (54°C) was 23°C lower than the native starch gelatinization endotherm peak (77°C). For each initial heating temperature for *du wx* starch, retrogradation enthalpy increased with storage time (Fig. 2). The maximum of retrogradation enthalpy after one day was ≈ 10 J/g. After 22 days, it was ≈ 15 J/g for all initial heating temperatures above T_c . Typical thermograms of retrogradation for *du wx* starch after heating to different initial heating temperatures after 12 days of storage at 4°C are shown in Fig. 3.

The retrogradation enthalpy of *su2 wx* starch also increased with the storage time for each initial heating temperature, but for all initial heating temperatures the retrogradation enthalpy of *su2 wx* starch was far lower (Fig. 4) than for *du wx* starch. After one day of storage, no retrogradation endotherm was observed in any DSC thermogram of *su2 wx* starch, and thus the data are not plotted in Fig. 4. The maximum of retrogradation enthalpy increased from 1.1 J/g after three days to 7.0 J/g after 30 days. The maximum appears for initial heating to 80°C, approximately the completion temperature of gelatinization. Figure 5 shows typical thermograms of retrogradation for *su2 wx* starch as a function of different initial heating temperatures after 30 days of storage at 4°C. The retrogradation endotherm occurs in a similar temperature range for *su2 wx* starch and *du wx* starches; however, the retrogradation enthalpy of *du wx* starch is a higher proportion of gelatinization enthalpy than for the *su2 wx* starch for the time scale of this work.

It is clear from Figs. 2 and 4 that retrogradation of each starch was influenced by the initial heating temperature. Within the gelatinization temperature range, the retrogradation enthalpy increased as the initial heating temperature increased. The maximum retrogradation enthalpy for each starch was for an initial heating temperature approximately corresponding to the native gelatinization endotherm T_c . For *du wx* starch, retrogradation enthalpy after one and three days of storage was similar between 85 and 105°C and then decreased as initial heating temperature increased. After 12 days, the enthalpy was nearly independent of initial heating temperature in the range from 85 to 180°C (Fig. 2). However, the retrogradation of *su2 wx* starch decreased sharply as the initial heating temperature increased above the gelatinization temperature range, even for storage as long as 30 days (Fig. 4). After 30 days of storage, the retrogradation enthalpy of *su2 wx* starch was 2.1 J/g for starch initially heated to 150°C and only 0.4 J/g for starch initially heated to 180°C.

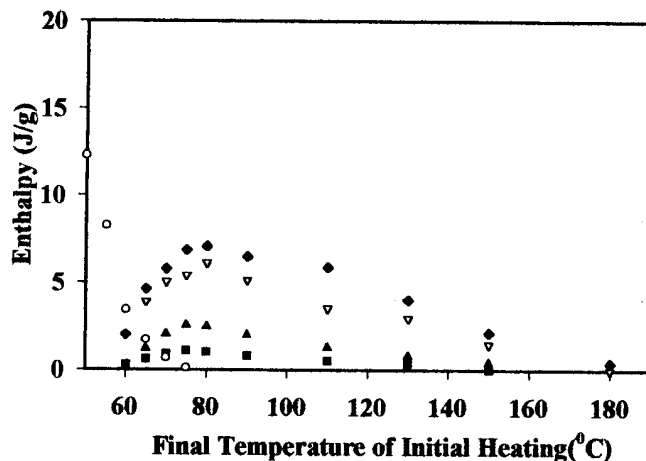


Fig. 4. Retrogradation of 30% *su2 wx* gelatinized starch as a function of initial heating temperature. Residual gelatinization enthalpy immediately after initial heating (\circ). Retrogradation enthalpy was measured after three days (\blacksquare), seven days (\blacktriangle), 20 days (∇), and 30 days (\blacklozenge).

For initial heating to temperatures within the gelatinization temperature range, no retrogradation endotherm was observed on immediate reheating for *du wx* and *su2 wx* starches. On storage after heating to temperatures in this range, retrogradation enthalpy increased as initial heating temperature increased. Furthermore, for *du wx* starch retrogradation continued to develop as storage time increased even after 12 days (Fig. 2). For *du wx* starch, the relationship between the retrogradation enthalpy (after 12 days) and lost gelatinization enthalpy is shown in Fig. 6. A linear gain (slope ≈ 1) in the retrogradation enthalpy was observed after the first 4 J/g lost. This analysis could not be applied to *su2 wx* starch due to the overlap of retrogradation and residual gelatinization endotherms for the 55 and 60°C initial heating temperatures.

Kinetics of *du wx* Starch Retrogradation

In addition to the retrogradation enthalpy data described for one, three, seven, 12 and 22 days, data for additional longer (40 and 70 days) and shorter storage times (less than one day) of *du wx* starch were obtained as well for selected initial heating temperatures. For initial heating to 85°C, retrogradation was first observed at 2.5 hr (Fig. 7). For initial heating to 120°C, retrogradation enthalpy was first observed at 4 hr (data not shown); for initial heating to 140°C and 160°C, retrogradation was first observed at 6 hr (data not shown) and 15 hr (Fig. 8), respectively; and for initial heating to 180°C, retrogradation was first observed at 33 hr (data not shown). No difference was observed for samples stored for 40 and 70 days for any initial heat treatment $\geq 85^\circ\text{C}$ (data not shown). Consequently the enthalpies of all the samples stored for 40 and 70 days were combined to calculate the mean to represent the complete retrogradation enthalpy ($\Delta H_L = 16.2 \pm 0.2$ J/g, mean \pm standard deviation for 10 samples) for purposes of the Avrami analysis. For initial heating temperatures $\leq 140^\circ\text{C}$, there appeared to be two different linear regions of the Avrami plot: for storage less than one day and for storage more than one day (Fig. 9). Consequently, two different Avrami indices (n) and rate constants (k) were calculated for starch retrogradation after initial heating to temper-

atures of 85–140°C. The calculated parameters from the regression analyses are shown in Table II. For storage longer than one day, the Avrami index (n) increased with initial heating temperature and was much larger for starches heated to 160 or 180°C (1.17 and 1.24). For storage longer than one day, the crystallization rate constant (k) decreased as initial heating temperature increased. For storage less than one day, the Avrami index (n) increased and the rate constant (k) decreased as initial heating temperature went from 85 to 140°C.

DISCUSSION

Retrogradation of *du wx* and *su2 wx* Starches After Heating to Initial Temperatures Within and Above the Gelatinization Temperature Range

Starch retrogradation increased as the initial heating temperature increased within the gelatinization temperature range for *du wx* and *su2 wx* starches. A gain in retrogradation enthalpy equivalent to the lost gelatinization enthalpy was observed for lost gelatinization enthalpy between 4 and 18 J/g for *du wx* starch (Fig. 6). A similar proportional relationship was previously observed for retrogradation of *wx* starch when lost gelatinization enthalpy was >6 J/g (Fisher and Thompson 1997).

For *du wx* starch after initial heating temperatures within the gelatinization temperature range, the retrogradation endotherm peaks appeared at the same temperatures, suggesting that newly formed structures are the same for all initial heating temperatures. Retrogradation is thought to result from transformation from the random coil state to a double helix, and subsequent association of these double helices (Gidley et al 1990). The broad endotherm and lower temperature for retrogradation compared to gelatinization indicates the new starch structures are less well ordered than the native starch structure. The residual gelatinization peak for samples heated to temperatures within the gelatinization temperature range (Figs. 3 and 5) occurs at a higher temperature as initial heating temperature increases. This observation for *du wx* and *su2 wx* starches might be due partly to preferential gelatinization of the population of more readily gelatinized granules and partly due to annealing of remaining ungelatinized granules (Fisher and Thompson 1997).

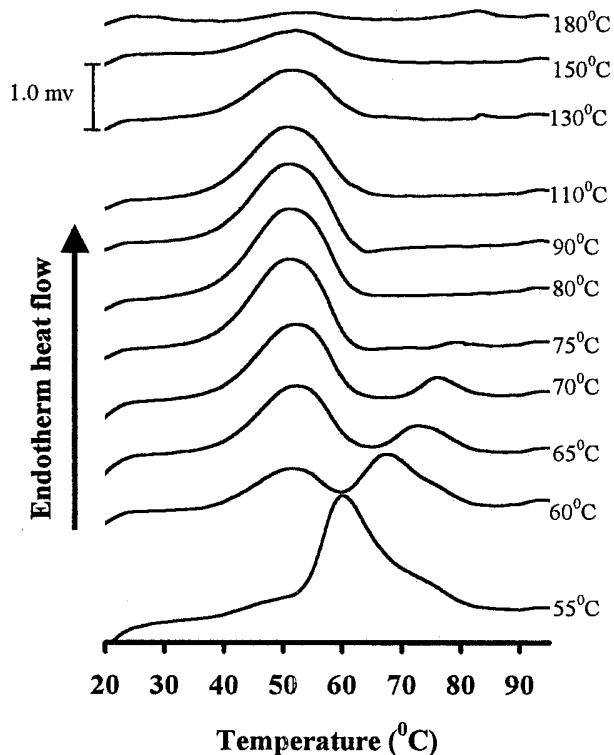


Fig. 5. Typical thermograms of retrogradation for 30% *su2 wx* starch with different initial heating temperatures and storage for 30 days at 4°C. Labeling refers to initial heating temperature.

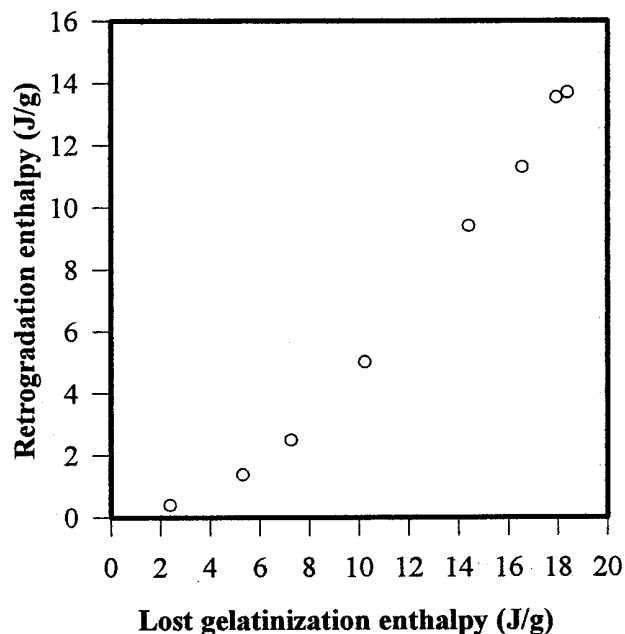


Fig. 6. Lost gelatinization enthalpy vs. retrogradation enthalpy of 30% *du wx* starch after 12 days of storage after different initial heating temperatures. Lost gelatinization enthalpy = native gelatinization enthalpy (18.4 J/g) – residual gelatinization enthalpy.

That the retrogradation enthalpy was significantly different between *du wx* and *su2 wx* starches may be related to different gelatinization behaviors. Residual gelatinization of *su2 wx* starch was more sensitive to holding at an initial heating temperature than was *du wx* starch (Table I). For *su2 wx* starch, the proximity of the retrogradation endotherm and the residual gelatinization endotherm led to some overlap (see Fig. 5, initial heating to 55 and 60°C). For the 65°C treatment, the difference in the peak temperatures for the retrogradation endotherm and residual gelatinization endotherm is 7.5°C less than for *du wx* starch. The *su2 wx* starch also has an unusually low T_o , T_p , and ΔH for native starch gelatinization (Table I). Inouchi et al (1991a) reported similar gelatinization enthalpy for this starch. From the observed lower gelatinization enthalpy and temperature for *su2 wx* starch, Inouchi et al (1991a) assumed that the starch had low crystallinity and less hydrogen bonding. Our results for *su2 wx* starch indicate that when *su2 wx* starch was heated to just below T_o of gelatinization and held 20 min, not only did the T_o shift to the higher temperature, but the enthalpy decreased greatly (Table I). Based on the different susceptibility to heating to just below T_o , it is likely that the amylopectin structure (chain length) or the granule structure (the proportion of crystalline regions and amorphous regions) are different despite the same X-ray diffraction pattern (A-type) for *su2 wx* and *du wx* starches. The different effect of initial heat treatment on gelatinization behavior may be related to the slower retrogradation rate and small retrogradation enthalpy for *su2 wx* starch.

Above the gelatinization temperature range, the effect of initial heating temperature on retrogradation was also different for these two starches. For *su2 wx* starch, at all storage times, the retrogradation enthalpy decreased as initial heating temperature increased.

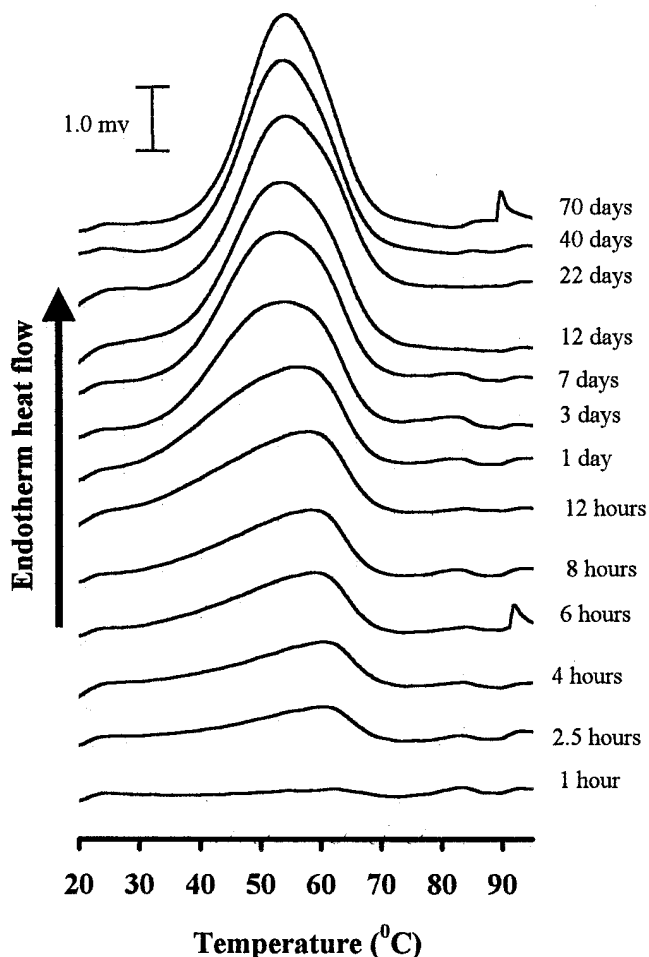


Fig. 7. Retrogradation of 30% *du wx* starch after initial heating to 85°C as a function of storage time. Labeling refers to storage times.

Even after 20 or 30 days of storage, a maximum retrogradation enthalpy was reached for the starch initially heated to around T_c of the gelatinization endotherm ($T_c \approx 80^\circ\text{C}$). On the other hand, the difference in retrogradation for *du wx* starch for initial heating temperatures at 85–105°C was quite small even for one day of storage (Fig. 2). After 12 days of storage, the retrogradation enthalpy was almost constant for all initial heating temperatures. Thus for *du wx* starch, different initial heating temperatures resulted in different extents of retrogradation in the early storage periods, but for long-term storage, the effect of different initial heating temperature was lost. The maximum retrogradation enthalpy was 16.2 J/g for all initial heating temperatures above T_c . Inouchi et al (1991b) observed a constant retrogradation enthalpy for ≥ 10 days of storage for *du wx* starch after gelatinization of native starch by DSC at a heating rate of 2°C/min to an unspecified final temperature.

Keetels et al (1996) anticipated that a higher degree of disorder, or higher extent of disentanglement, of starch molecules would result from a gelatinization at a higher temperature. Swinkels (1985) reported that starch granules cooked at $\approx 95^\circ\text{C}$ for 1 hr may still contain highly swollen hydrated starch aggregates. At a temperature $>100^\circ\text{C}$, the degradation to small parts or fragments, or even molecular depolymerization, may occur. The retrogradation data for *du wx* starch provide no evidence of depolymerization for initial heating to 180°C because the retrogradation enthalpy was the same as that of lower initial heat treatment was observed after 12 days of storage. However, for *su2 wx* starch, after initial heating to the higher temperature, the lower retrogradation enthalpy after long storage times could be due to either the depolymerization of starch molecules or a reduced retrogradation rate. Because significant depolymerization of *du wx* starch is unlikely, it would be unlikely for *su2 wx* starch as well.

Kinetics of *du wx* Starch Retrogradation

As shown in Figs. 2 and 4, for both *su2 wx* and *du wx* starches, the retrogradation was greatly influenced by storage time and dif-

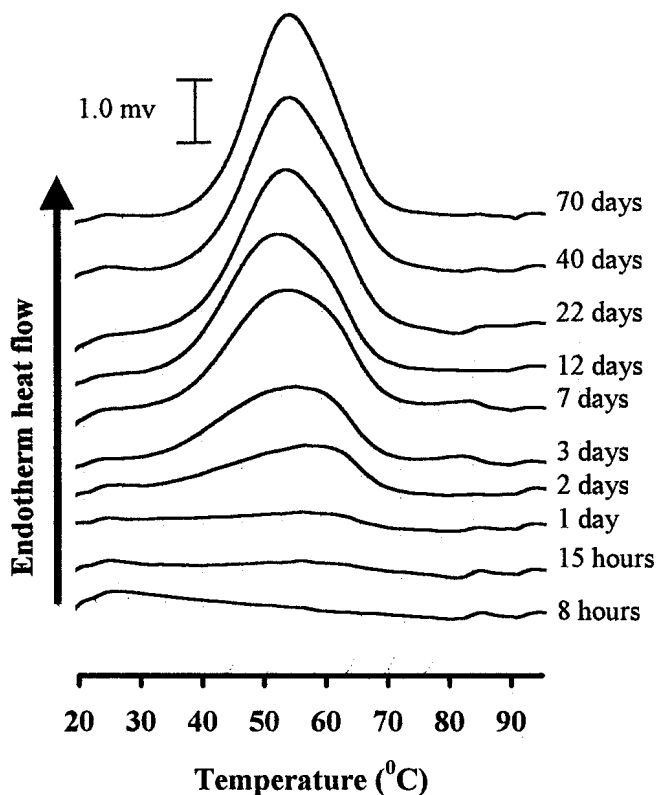


Fig. 8. Retrogradation of 30% *du wx* starch after initial heating to 160°C as a function of storage time. Labeling refers to storage times.

ferent gelatinization heat treatments. Fisher and Thompson (1997) observed a similar behavior for *wx* starch, but retrogradation of *ae wx* starch appeared complete after one day. Retrogradation of *du wx* starch was observed within one day for all initial heating temperatures except for 180°C. The retrogradation rate varied with different initial heating treatments. Retrogradation enthalpy (>1 J/g) was first observed at 2.5, 4, 6, 15 and 33 hr for initial heating temperature of 85, 120, 140, 160 and 180°C, respectively. Retrogradation of *du wx* starch occurred more rapidly for the lower initial heating temperatures ($\leq 140^\circ\text{C}$) than for the higher initial heating temperatures ($\geq 160^\circ\text{C}$). These results suggest that the initial heating temperature is a very important factor for the retrogradation of *du wx* starch. Thus *du wx* starch is a convenient sample for kinetic analysis. We suggest that results may be generalized to provide insight into retrogradation of other amylopectins.

As shown in Fig. 7, the retrogradation peak temperature for *du wx* starch initially heated to 85°C was shifted from 60°C, on storage for one day or less, to 55°C after more than one day. We suggest the formation of relatively longer double helices may dominate retrogradation within one day. A lower peak temperature of the endotherm after one day may indicate the association of more numerous shorter double helices. For *du wx* starch subjected to a higher gelatinization heating temperature (160°C), the shift in peak temperature was delayed, and occurred sometime between one and seven days (Fig. 8). The different timing of this shift reflects the overall slower retrogradation for initial heating to high temperature. Perhaps the higher initial heating temperature led to a greater disorientation of branch chains, thus slowing the formation of double helices.

The Avrami plots (Fig. 9) suggest two Avrami indices and rate constants best describe the retrogradation after heating to initial heating temperatures $\leq 140^\circ\text{C}$. At initial heating temperature $\geq 160^\circ\text{C}$, only one Avrami index and rate constant was apparent in the experimental time scale. One may suggest fitting one linear regression equation for initial heating at temperatures $\leq 140^\circ\text{C}$ as well, but from Table II, it can be seen that when all values are included for the regression analysis, a poorer fit results.

In this study, the utility of the Avrami parameter is to provide a basis for comparing kinetic results between different gelatinization heat treatments. For region II, starch heated to a lower temperature has a higher rate constant and lower Avrami index (Table II). The lower the temperature of the gelatinization heat treatment, the more rapid the retrogradation. The diverse values obtained for the Avrami index (0.30–2.27) suggest that the mechanism of starch retrogradation differs for different gelatinization heat treatments.

By fitting data for the entire storage period, Inouchi et al (1991b) reported 0.36 for the Avrami index for *du wx* starch (33%) after a gelatinization heat treatment “several degrees higher” than T_c (the exact temperature was not reported). They concluded that the retrogradation mechanism of *du wx* starch was not well described by the theory of Avrami. Early workers (McIver et al 1968, Colwell et al 1969, Wong and Lelievre 1982) reported a value of 1.0 for the Avrami index for wheat starch and

concluded that instantaneous nucleation and one-dimensional growth accounted for starch crystallization during retrogradation. The present report shows various Avrami indices (0.30–1.24 in Region II) for the different gelatinization heat treatments, indicating the crystallization mechanism for the *du wx* starch retrogradation may be dependent on the initial heating temperature. Furthermore, the two different slopes and intercepts due to two linear regions suggest that retrogradation of *du wx* starch heated to $\leq 140^\circ\text{C}$ results from two processes. The first process may be the formation of the relatively longer double helices, as within one day, the retrogradation endotherm shows a higher peak temperature (Fig. 7). The second process may involve formation of other double helices and the association of double helices. The present report suggests that the use of the Avrami equation to predict the mechanism of crystallization in retrogradation as done in previous studies (McIver et al 1968, Colwell et al 1969, Wong and Lelievre 1982) is not straightforward.

The assumption that the measured parameter, retrogradation enthalpy, was linearly related to crystallinity may not always be true for starch. Starch retrogradation may be due to two processes: the formation of double helices and the association of double helices. Crystallinity of starch is understood as associated double helices. For the gelatinization of granular starch, Cooke and Gidley (1992) suggested the primary determinant of endotherm

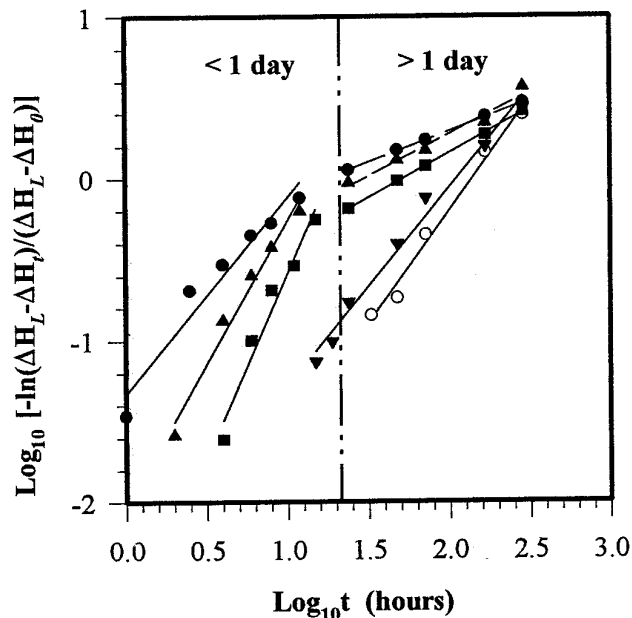


Fig. 9. Fit of Avrami equation for 30% *du wx* starch retrogradation after different initial heating temperatures: 85°C (●), 120°C (▲), 140°C (■), 160°C (▼) and 180°C (○). Two linear regions were observed for length of storage (<1 day, >1 day).

TABLE II
Avrami Index and Rate Constant for Retrogradation of *du wx* Starch Initially Heated to Various Initial Heating Temperatures^a

Initial Heating Temperature (°C)	All Times			Region I ^b			Region II ^b		
	<i>k</i>	<i>n</i>	<i>r</i> ²	<i>k</i>	<i>n</i>	<i>r</i> ²	<i>k</i>	<i>n</i>	<i>r</i> ²
85	1.02×10^{-1}	0.59	0.86	4.31×10^{-2}	1.20	0.95	3.88×10^{-1}	0.30	0.99
120	5.12×10^{-2}	0.71	0.85	8.67×10^{-3}	1.75	0.98	2.56×10^{-1}	0.37	0.99
140	2.45×10^{-2}	0.83	0.86	1.21×10^{-3}	2.27	0.97	1.17×10^{-1}	0.51	0.99
160	2.82×10^{-3}	1.17	0.96	nc ^c	nc	...	2.82×10^{-3}	1.17	0.96
180	1.77×10^{-3}	1.24	0.98	nc	nc	...	1.77×10^{-3}	1.24	0.98

^a Data subjected to regression analysis to determine best-fit values of *k* (rate constant), *n* (Avrami exponent), and correlation coefficient (*r*²) are shown in Fig. 8.

^b For initial heating to $\leq 140^\circ\text{C}$, Region I corresponds to storage for <1 day and Region II corresponds to storage for >1 day. For initial heating to 160°C, all data were subjected to a single linear regression analysis. For 180°C, no retrogradation could be observed at <1 day (Fig. 9).

^c Not calculated, insufficient retrogradation data for <1 day.

enthalpy is melting of double helices and not disruption of their packing in crystalline array. Thus, for crystalline retrograded starch, the retrogradation enthalpy may correspond more to the melting of double helices than to loss of crystallinity. Moreover, double helical material not in crystalline array would also be observed by DSC. Noncrystalline double helices may occur on retrogradation because the double helices are not completely free to move into crystalline register: the branched molecules in starch may be a constraint to crystalline ordering during retrogradation. Cameron et al (1994) have suggested that the double helices from the outer chains in the amylopectin molecules do not aggregate to develop crystallinity. For these reasons, it may not be reasonable to predict a precise crystallization mechanism by fitting the Avrami equation to starch retrogradation data obtained by DSC. However, the fitting of the Avrami equation in two regions from the enthalpy change during the retrogradation process gives insight into the observed differences in retrogradation rate for starch heated to various initial temperatures. Retrogradation kinetics of *du wx* starch is affected by different gelatinization heat treatments.

CONCLUSIONS

Starch retrogradation increases when increasing the initial heating temperature within the gelatinization temperature range for *du wx* and *su2 wx* starches. An initial heating temperature corresponding to the completion point of gelatinization results in the greatest retrogradation enthalpy for the both starches for short storage times. The retrogradation of *du wx* starch is more rapid than that of *wx* and *su2 wx* starches. When heated to a temperature above the gelatinization range, the retrogradation of *du wx* starch is independent of heating treatment after 12 days of storage, in contrast to *su2 wx* starch. The extent of retrogradation is far lower for *su2 wx* starch than for *du wx* starch. For *du wx* starch, the lower the initial heating temperature, the higher the rate constant and the lower the Avrami index. The retrogradation kinetics of *du wx* starch are greatly influenced by different gelatinization heat treatments.

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