

Correlation Between Starch Retrogradation and Water Mobility as Determined by Differential Scanning Calorimetry (DSC) and Nuclear Magnetic Resonance (NMR)

Yu-shiun Lin,¹ An-I Yeh,¹ and Cheng-yi Lii¹⁻³

ABSTRACT

Cereal Chem. 78(6):647-653

Starches from nine varieties of rice, including four indica, three japonica, and two waxy cultivars, were used for the investigation of the correlation between retrogradation and water mobility. Retrogradation and water mobility were analyzed by differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR) and expressed as enthalpy change (ΔH) and differential relaxation rate (ΔR_2) for water-¹⁷O. Water contents were measured by DSC and Karl-Fischer methods. The results indicated that three different profiles, based on amylose content, were observed for the ΔH changes of rice starch cooks during storage. They fit well to the nonlinear regression equations of exponential rise to

maximum and exponential growth models. The water content, as measured with DSC, decreased during storage but increased as measured with the Karl-Fischer method. This discrepancy might be attributed to the different characteristics of water measured by the two methods. The ΔR_2 of rice starch cooks showed an increasing trend during storage but was more complicated than the ΔH trend. The nonlinear regression models were also applied to fit the changes of ΔR_2 for indica varieties in the initial six days and for waxy varieties up to 24 days. This resembled the ΔH changes.

Starchy food is usually heated in water and often stored before being consumed. Retrogradation of gelatinized starch molecules is initiated during storage. This process has been extensively investigated using differential scanning calorimetry (DSC), X-ray diffraction, and rheological techniques to monitor the changes at molecular or macroscopic level (Ring et al 1987; Joupila et al 1998; Vodovotz and Chinachoti 1998; Yuan and Thompson 1998).

The Avrami equation is most frequently used to analyze the kinetic behavior of starch recrystallization (Longton and LeGrys 1981; Russell 1987; Baik et al 1997; Fan and Marks 1998; Joupila et al 1998; Liu and Thompson 1998). The equation is expressed as $\Theta = 1 - \exp(-kt^n)$ where Θ is % crystallized starch at time t ; k is a rate constant; and n is the Avrami exponent related to nucleation and crystal growth processes. Additionally, an exponential first-order equation is used in studies to estimate the rate constant when a certain mode of nucleation and growth is assumed (Russell and Oliver 1989; Zhang and Jackson 1992). However, because the starch system is more complicated than that of ordinary polymers, the fit of these equations may be inappropriate. Thus, conclusions should be very cautiously drawn from empirical data (Slade and Levine 1986; Zhang and Jackson 1992; Liu and Thompson 1998).

Water content is one of the most influential factors in starch retrogradation and can affect the extent of crystallization (Longton and LeGrys 1981; Zelezak and Hosney 1986; Joupila et al 1998) through water diffusion (Hosney 1984) and plasticization (Slade and Levine 1986). However, because bound water has properties different from those of bulk water in the same system and various degrees of water "boundness" exist, the function of water in both categories during aging becomes uncertain if only the water amount is restricted.

The bound water has been commonly analyzed by DSC, differential thermal analysis (DTA) or dynamic mechanical analysis (DMA) (Bushuk and Mehrotra 1977; Wynne-Jones and Blanshard 1986; Li et al 1998; Vodovotz and Chinachoti 1998). More detailed information such as dynamic behavior or water distribution can be also determined using nuclear magnetic resonance (NMR) spectro-

scopy (Kim-Shin et al 1991; Lai et al 1998) or magnetic resonance imaging (MRI) (Ruan et al 1996). NMR has long been used to study dynamic characteristics of the hydration and mobility of water in starch suspensions (Richardson et al 1987), starch gels (Wynne-Jones and Blanshard 1986; Li et al 1998) or paste (Lai et al 1998), and bread (Wynne-Jones and Blanshard 1986; Kim-Shin et al 1991).

The change in starch at the molecular level and water mobility changes during storage have been discussed individually (Wynne-Jones and Blanshard 1986; Lai et al 1998). However, concurrent study of both properties has seldom been reported. Hence, this investigation on the correlation between the retrogradation enthalpy and water mobility of different rice starches was initiated.

MATERIALS AND METHODS

Nine varieties of milled rice (first crop of 1996), obtained from the Taichung District Agricultural Improvement Station, Changhua, Taiwan, were used as samples. They were four indica (Kaoshiung Sen 7 [KSS7], Taichung Native 1 [TCN1], Taichung Sen 10 [TCS10], Taichung Sen 17 [TCS17]), three japonica (Taichung 189 [TC189], Taigeng 9 [TG9], TaiNung 67 [TNU67]) and two waxy varieties (Taichung Waxy 70 [TCW70], Taichung Sen Waxy 1 [TCSW1]). Rice starches were isolated using the modified alkaline steeping method (Yang et al 1984) and apparent amylose contents were determined as described by Chrastil (1987).

A 25% rice starch suspension (100 mg, w/w) kept ≈ 1 hr at room temperature for water equilibrium was put into a stainless steel crucible. The crucible was sealed using a stainless steel cover and an aluminum O-ring. The sample crucible was heated in a boiling-water bath for 30 min, then cooled at room temperature for 30 min, followed by storage at 4°C for the retrogradation study. The melting behavior of retrograded starch was examined by a differential scanning calorimeter (Setaram DSC121, Caluire Cedex, France) at different storage times. Samples were scanned from 5 to 130°C at 5°C/min with an empty crucible used as reference.

A spectrometer (MSL-200, Bruker Co., Germany) operated at 27.13 MHz was used for the ¹⁷O NMR R_2 measurement. Natural abundance ¹⁷O measurements were applied during the investigation. A 90° single-pulse for ¹⁷O of pulse width 13 μ sec and a recycling time of 400 msec was used. The broadband proton decoupling was also performed. The starch cook was prepared in a 10-mm NMR tube using the same preparation procedure as for the DSC sample. The NMR tube was spun at 12 ± 1 Hz and measurement was done in triplicate at $30 \pm 1^\circ\text{C}$ with 1,200 scans.

¹ Graduate Institute of Food Science and Technology, National Taiwan University, Taipei 106, Taiwan.

² Institute of Chemistry, Academia Sinica, Nankang, Taipei 115, Taiwan.

³ Corresponding author. Phone: 886-2-2789-8568. Fax: 886-2-2783-1237. E-mail: cylie@chem.sinica.edu.tw

RESULTS AND DISCUSSION

Apparent Amylose Content

The apparent amylose contents of rice starches are listed in Table I. They were 23.8–26.1% for indica, except for TCS10; 11.4–14.1% for japonica and TCS10; and ≈1% for waxy varieties.

Starch Retrogradation

The degree of retrogradation for the 25% rice starch cook was measured as the enthalpy of melting of recrystallized starch (ΔH). Three different profiles of the retrogradation process were observed when the retrogradation enthalpy was plotted versus storage time (Fig. 1). For starches with high amylose contents of indica varieties (Fig. 1a), the ΔH was increased rapidly in the early stage, then reached the plateau. For the intermediate amylose contents of TCS10 (Fig. 1a) and japonica varieties (Fig. 1b), the ΔH did not change significantly in the first two days but rose steadily as the storage time prolonged. The ΔH of waxy varieties did not alter for

In ^{17}O NMR analysis, the line width at half-height of frequency domain spectrum of pure water ($\Delta\nu_{\text{free}}$) was subtracted from that of the sample ($\Delta\nu_{\text{obs}}$) to correct for any residual magnetic field inhomogeneity. The differential transverse relaxation rate ($\Delta R_2/\text{sec}$) was then obtained by the equation: $\Delta R_2 = \pi(\Delta\nu_{\text{obs}} - \Delta\nu_{\text{free}})$ (Richardson et al 1987; Lai et al 1998).

A calorimeter (Setaram DSC 121) according to the melting mode was used to measure freezable water (Bushuk and Mehrotra 1977). The sample crucible was prepared in the same manner as in the retrogradation investigation, followed by quenching with liquid nitrogen before measurement. The sample was heated from -25 to 20°C at a rate of $1^\circ\text{C}/\text{min}$. The melting endothermic peak of ice at $\approx 0^\circ\text{C}$ was recorded, and the melting enthalpy of deionized water was used as a reference (Chung and Lee 1991).

The water content in starch cook, as prepared above, was determined with the Karl-Fischer titration method (DL18 Karl Fischer titrator, Mettler-Toledo AG, Switzerland). During the determination, Hydranal solvent consisting of sulfur dioxide and imidazole in methanol, and Hydranal titrant containing iodine (RdH Laborchemikalien GmbH & Co. KG, Seelze, Germany) were used. The water content was displayed automatically when the titration was completed.

A nonlinear regression analysis was applied to obtain the time course of retrogradation enthalpy and of differential transverse relaxation rate changes. Built-in equations in SigmaPlot library, exponential rise to maximum $y = y_0 + a(1 - e^{-bx})$ and exponential growth $y = ae^{bx}$, were selected to fit the data from DSC and NMR by using SigmaPlot software (v. 4, SPSS, Inc., Chicago, IL). The former was applied to those data with convergent change and the latter to those without convergence. The independent variable (x) was assigned to the storage time (t) in this study, and the dependent variable (y) was assigned to ΔH and ΔR_2 . Regressions were implemented automatically by the software to find the values of y_0 , a and b . Hence, the equation could most closely describe the data ($P < 0.0001$) using the values of the independent variable to predict the value of a dependent variable.

The starch cook was also examined by scanning electron microscopy (JSM-5400, Jeol Co., Japan) operated at 20 kV. The freeze-dried samples were mounted on the stubs and sputter-coated with gold for examination.

Statistical Analysis

Differences in the water contents of starch cooks during storage were analyzed by the analysis of variance (ANOVA, general linear model) and Duncan's new multiple range test ($P < 0.05$). Pearson correlation analysis was computed for the coefficient between the average value of apparent amylose content and the exponent of the nonlinear regression equation. All statistical analyses were conducted with SAS statistical analysis software (SAS Institute, Cary, NC).

TABLE I
Apparent Amylose Contents and Melting Temperatures of Retrogradation Transition of Rice Starches

Variety	Apparent Amylose (%)	Retrogradation Temperature ($^\circ\text{C}$) ^a		
		T_0	T_p	T_c
Indica				
KSS7	25.93 ± 0.68	38.43 ± 0.09	52.59 ± 0.31	65.92 ± 0.73
TCN1	23.81 ± 0.17	37.78 ± 0.09	50.16 ± 0.01	64.11 ± 0.27
TCS17	26.13 ± 1.57	37.47 ± 0.11	49.80 ± 0.10	63.59 ± 0.29
TCS10	11.39 ± 0.37	37.66 ± 0.12	51.60 ± 0.14	62.93 ± 0.23
Japonica				
TC189	14.06 ± 0.21	37.60 ± 0.19	51.16 ± 0.32	63.12 ± 0.21
TG9	12.65 ± 0.20	37.45 ± 0.20	51.03 ± 0.18	62.42 ± 0.04
TNu67	13.12 ± 0.10	37.05 ± 0.17	50.97 ± 0.39	62.69 ± 0.35
Waxy				
TCW70	0.92 ± 0.09	36.72 ± 0.19	50.65 ± 0.03	62.56 ± 0.14
TCSW1	0.97 ± 0.00	37.25 ± 0.25	51.26 ± 0.33	62.93 ± 0.22

^a Retrogradation transition at 30 days; T_0 , T_p , T_c = onset, peak, and completion temperatures of retrogradation transition.

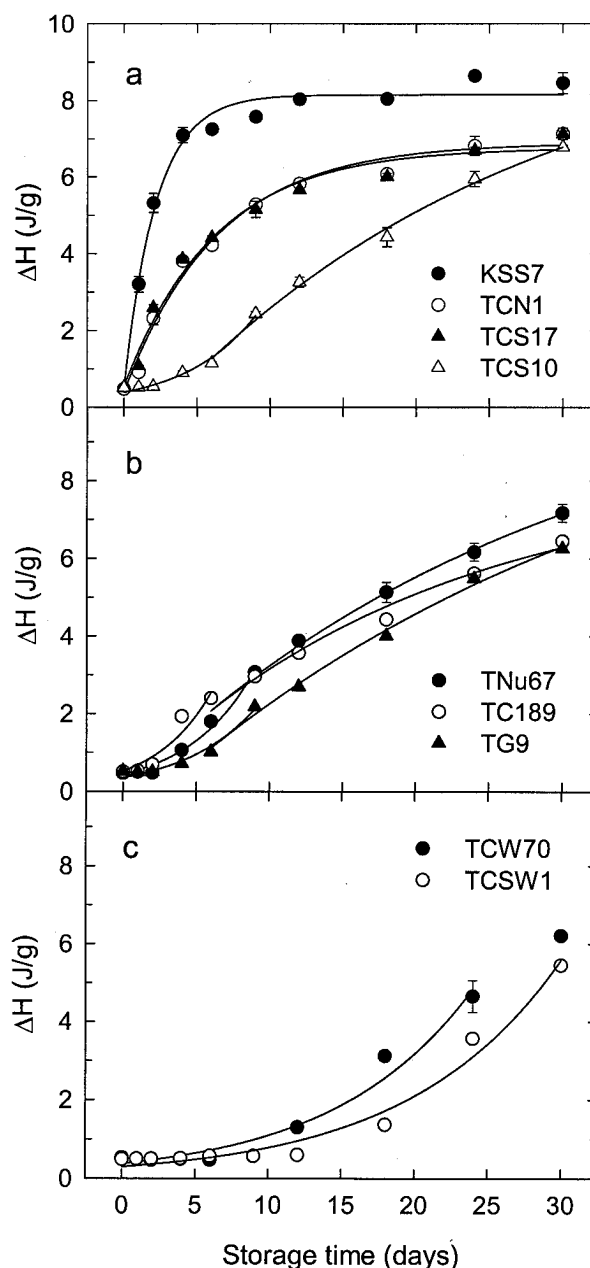


Fig. 1. Retrogradation enthalpy change (ΔH) of different rice starch cooks during storage (a, indica; b, japonica; c, waxy).

9–12 days, then it started to increase (Fig. 1c). Generally, the ΔH tended to be related to the amylose content of starch up to 30 days. However, it should be noted that the enthalpies of TCS10, japonica, and waxy varieties did not reach the plateau during 30 days of storage, which suggested that starch recrystallization was still in progress and the ΔH would continue to rise.

The retrogradation transitions of rice starches occurred at 36–66°C (Table I). This was similar to other varieties of rice (Chang and Liu 1991; Fan and Marks 1998), wheat (Longton and LeGrys 1981; Ring et al 1987), and maize starches (Yuan et al 1993). This endotherm was regarded as the melting of the retrograded amylopectin (Zeleznaek and Hosenev 1986; Ring et al 1987; Russell 1987; Yuan et al 1993) and therefore, would be affected by the amount and the molecular characteristics of amylopectin. The retrogradation was accelerated by the amylopectin with longer average chain length (Kalichevsky et al 1990; Yuan et al 1993). Shi and Seib (1992) indicated the retrogradation of waxy starches was directly proportional to the mole fraction of branches with DP 14–24, and inversely proportional to the mole fraction of branches with DP 6–9. The high ratio of branches with DP 20–30 (Yuan and Thompson 1998) or DP ≥ 35 (Sasaki and Matsuki 1998) was also reported to increase the retrogradation enthalpy. The indica rice with high amylose content used in this investigation carries extra long chain branches (DP > 100) in the amylopectin (Lu et al 1997) which can increase retrogradation (Fig. 1a). The low degree of retrogradation for the waxy starches (Fig. 1c) was attributed to the high proportion of short chain branches of DP 6–9 (Lu et al 1997).

Modeling Study

Avrami equation is frequently applied to obtain the rate constant and the nature of nucleation and growth process for starch retrogradation (Russell and Oliver 1989; Zhang and Jackson 1992; Liu and Thompson 1998). One of the requirements for applying Avrami equation is that the recrystallization process reach the equilibrium state. Because only three samples satisfied this requirement (Fig. 1a), other non-linear regressions were applied to this investigation.

The regression results are excerpted in Table II. The equation of exponential rise to maximum was fitted for the expression of the retrogradation process on the starches with high amylose content ($r^2 \approx 0.99$); and the exponential growth for waxy starches ($r^2 \approx 0.97$). Because the retrogradation rate for TCS10 and japonica varieties were not high at early stage, the exponential growth model was applied in the first six to nine days, followed by the equation of exponential rise to maximum. In addition to the higher retrogradation rate of KSS7 starch, the statistical analysis also showed that the exponent of each equation for different models was linearly correlated with the amylose content. The correlation coefficient is

0.99 for exponential rise to maximum model ($P < 0.001$), and 0.97 for exponential growth model ($P = 0.0013$). These fitted profiles thus implied that the amylose content was one of the influential factors on starch retrogradation, as reported in the literature (Gudmundsson and Eliasson 1990; Chang and Liu 1991; Baik et al 1997; Fan and Marks 1998).

The rapid gelation of amylose and slow recrystallization of the short amylopectin branches during retrogradation does not proceed completely independently (Russell 1987; Gudmundsson and Eliasson 1990; Klucinec and Thompson 1999). Amylose is not only involved in the recrystallization of amylopectin but also functions as nuclei during recrystallization (Gudmundsson and Eliasson 1990). The rate, in the initial stage of nucleation controlled recrystallization process, was dependent on the amylose content (Baik et al 1997). This was confirmed by the result of the initial retrogradation rate in proportion to the amount of amylose content of the starch cook ($r = 0.69$, $P < 0.05$). The result also suggested, when amylose content was $\leq 24\%$, crystallization would be promoted and showed no lag period (Fig. 1a). The extra long chains of amylopectin might have a function similar to amylose. On the other hand, retrogradation of waxy rice starches would be delayed to a great extent, with little or no contribution from amylose (Fig. 1c).

As for TCS10 and japonica varieties, the nucleation rate was slower due to containing only a moderate amount of amylose and amylopectin with no extra long chains. Hence, the retrogradation

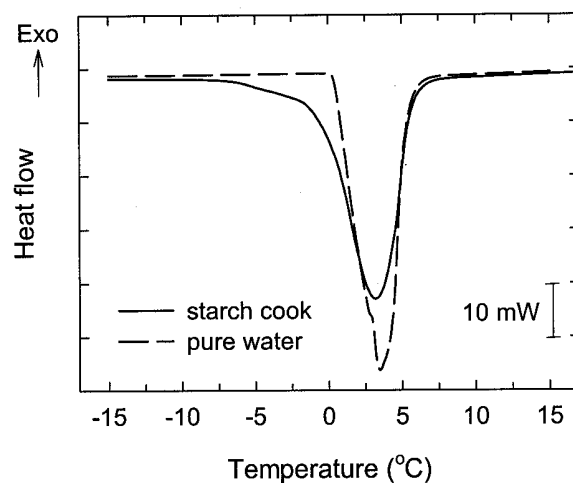


Fig. 2. Typical melting thermograms of ice crystals in pure water and starch cook.

TABLE II
Nonlinear Equations for Retrogradation Enthalpy Changes (ΔH , J/g) of Rice Starch Cooks as a Function of Time (t , days)

Variety	Equations ($\Delta H =$)	r^2	Initial Rate ^a	Asymptotic Value
Indica				
KSS7	$0.49 + 7.66 \times (1 - e^{-0.458t})$	0.987	3.508	8.15
TCN1	$0.39 + 6.51 \times (1 - e^{-0.156t})$	0.987	1.016	6.90
TCS17	$0.54 + 6.23 \times (1 - e^{-0.161t})$	0.983	1.003	6.77
TCS10	$0.38 \times e^{0.204t}$ ($t \leq 9$) $-1.14 + 11.42 \times (1 - e^{-0.040t})$ ($6 \leq t \leq 30$)	0.985 0.995	0.078	10.28
Japonica				
TC189	$0.52 \times e^{0.263t}$ ($t \leq 6$) $8.21 \times (1 - e^{-0.048t})$ ($6 \leq t \leq 30$)	0.910 0.981	0.137	8.21
TG9	$0.350 \times e^{0.201t}$ ($t \leq 9$) $-0.88 + 12.23 \times (1 - e^{-0.030t})$ ($6 \leq t \leq 30$)	0.973 0.995	0.070	11.35
TNu67	$0.43 \times e^{0.219t}$ ($t \leq 9$) $11.13 \times (1 - e^{-0.034t})$ ($6 \leq t \leq 30$)	0.985 0.994	0.094	11.13
Waxy				
TCW70	$0.38 \times e^{0.106t}$ ($t \leq 24$)	0.966	0.040	— ^b
TCSW1	$0.29 \times e^{0.098t}$	0.978	0.028	—

^a Expressed as $d(\Delta H)/dt$ at $t = 0$.

^b No convergence.

rate was slow, resembling that of waxy starch in the early stage, followed by increasing rapidly as that of high amylose starch (Fig. 1b). The asymptotic value of amylopectin retrogradation (Table II) calculated from the equations was in proportion to the amount (Gudmundsson and Eliasson 1990; Lu et al 1997) and the ratio of the branches with DP 14–24 of amylopectin (Shi and Seib 1992).

Freezable Water by DSC

The typical melting thermograms of ice crystals in pure water and starch cook are illustrated in Fig. 2. Frozen pure water melted right at 0°C with abrupt drop in endothermic peak. However, the ice in starch cook begins to melt gradually from ≈−10°C, indicating the existence of liquid water below subzero temperature. Such a melting curve could be thus divided into two parts, one for the free water with the same phase transition temperature as bulk water, and the other for the freezable bound water that has weak interaction with polymer chains and a phase transition <0°C (Giménez et al 1998).

Assuming the melting enthalpies of pure ice and ice in macromolecular systems are the same (Radosta and Schierbaum 1990), the total amount of freezable water calculated from DSC endothermic peak area was ≈63% in 25% starch cooks (Table III). The portion of endothermic peak <0°C was the freezable bound water at 5.2–6.8%, so the free water was ≈57%. The remaining 12% (75–63%) was unfreezable water that is assumed to associate closely with starch molecules (Giménez et al 1998; Li et al 1998). Among these rice varieties, the water contents measured by DSC were very close. These water contents decreased and the unfreezable water increased slightly as storage period was prolonged.

The X-ray diffraction pattern of the retrograded starch is B-type, where 36 water molecules are incorporated in the hexagonal unit cell of the crystallite (Sarko and Wu 1978; Leung et al 1983; Wynne-

Jones and Blanshard 1986; Imberty et al 1991). From the scanning electron micrograph of the retrograded sample (Fig. 3), the denser network structure of the starch cook was formed during storage. Water molecules should be partitioned from large domains into small domain inside the denser three-dimensional matrices and become unfreezable (Vodovotz and Chinachoti 1998). So both formation of B-type crystallites and denser structure of the retrograded starch cook lead to the increase of unfreezable water. However, the denser structure of TCW70 starch cook does not mean it contains more unfreezable water than KSS7 and TC189. Other factors such as the strength of water-starch interaction (Giménez et al 1998), the molecular weight, or distribution of polymers would also change the unfreezable water content (Radosta and Schierbaum 1990).

Water Content by Karl-Fischer Titration Method

Water content of KSS7, TC189, and TCW70 starch cooks determined by Karl-Fischer method are listed in Table IV. The values were 61–68%, lower than the initial water content (75%), and increased with storage time. During the measurement, water should be released from the sample before the titration process, which suggests different values with different samples are attributed to the varied water-starch interactions.

As compared with the freezable water content measured by DSC, the water content by Karl-Fischer was higher, except for fresh TC189 and the first two-day TCW70 starch cooks. The amount of free water calculated from Table III was ≈57%. So it can be concluded that the water content determined by Karl-Fischer included the free water and part of the freezable bound and unfreezable water. The high water content of KSS7 starch cook by Karl-Fischer may be due to the weak starch-water interaction.

During storage, despite the fact that some water is incorporated in the B-type crystallite and starch network, the unfreezable water

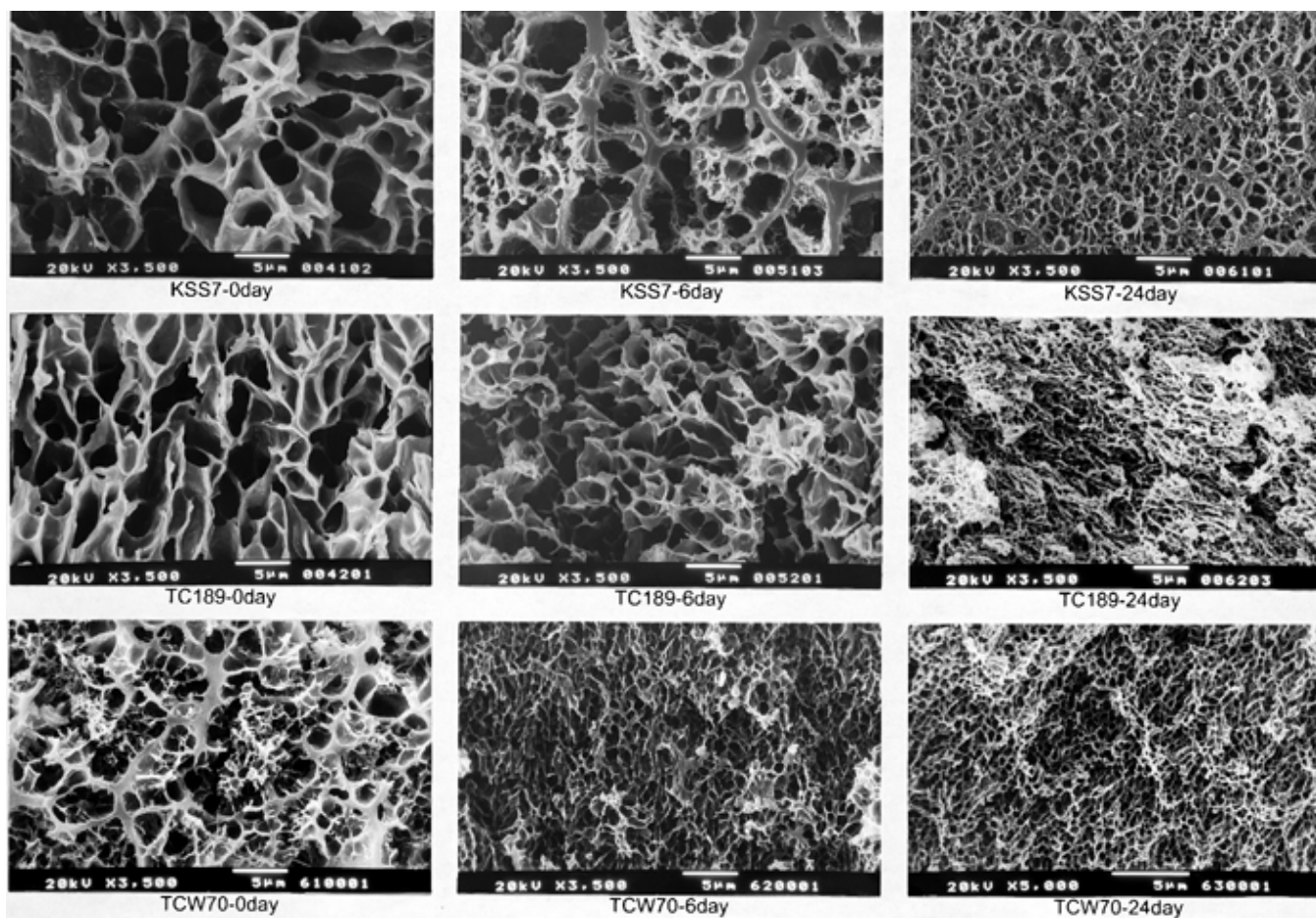


Fig. 3. Scanning electron micrographs of rice starch cooks during storage.

still remains in a metastable state and maintains high mobility (Li et al 1998). Furthermore, the free hydroxyl content of starch molecules should decrease due to the association of starch molecules (Vodovotz and Chinachoti 1998). Consequently, the interaction between water and starch should be weakened (Giménez et al 1998), and water should become more easily extracted by the Karl-Fischer reagent. This can explain the increase in water content as measured by Karl-Fischer as storage is prolonged.

¹⁷O NMR Relaxation Rate of Starch Cooks

The differential transverse relaxation rate (ΔR_2) can be used as an index for molecular mobility, with ΔR_2 inversely proportion to the mobility of the molecule. In this study, ¹⁷O NMR was used to investigate water mobility because the relaxation process of ¹⁷O nucleus was unaffected by the cross-relaxation experienced by the ¹H nucleus and the chemical exchange of ²H in water with ¹H in the matrix.

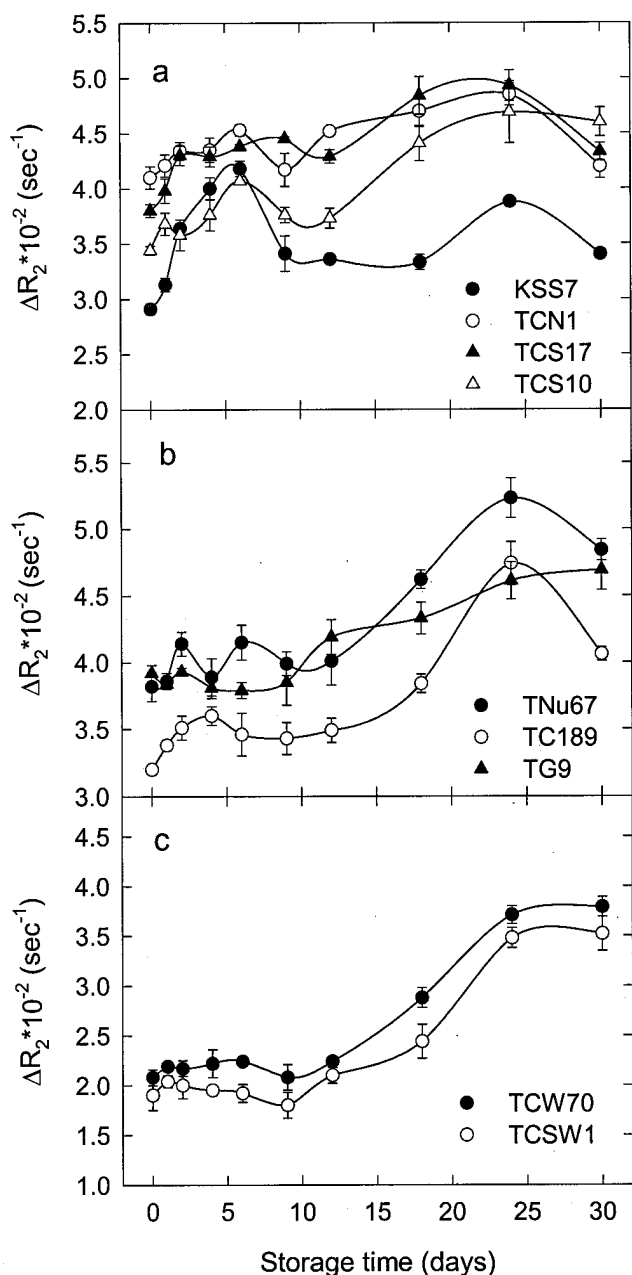


Fig. 4. Differential transverse relaxation rate for ¹⁷O nuclear magnetic resonance (NMR) of water molecules in 25% starch cook during storage (a, indica; b, japonica; c, waxy).

The ΔR_2 of ¹⁷O NMR for water molecules in 25% starch cooks during storage are shown in Fig. 4. The values of ΔR_2 for the freshly prepared samples varied. This implied that water mobility or water binding to starch molecules was not identical for the various starches. Compared with retrogradation enthalpy change, the profiles of ΔR_2 were more complicated, except for waxy starches, which did not change much in the early stage but then increased rapidly and leveled off.

Both nonlinear regressions mentioned previously were used to analyze the ¹⁷O NMR data. To show the relative change of ΔR_2 during storage, ΔR_2 of sample was divided by the value of ΔR_2 at $t = 0$ for normalization. The results are given in Table V. Due to the complicated profile of ΔR_2 change, only the data from the indica varieties (except TCS10) fit to the exponential rise to maximum model ($r^2 > 0.93$) in the initial six days (Fig. 5). However, only the data from the waxy varieties fit to the exponential growth model for the first 24-day period ($r^2 \approx 0.97$) and the TCS10 data for the first six-day period ($r^2 \approx 0.91$). The increase of ΔR_2 was much more rapid for KSS7 starch cook in the first six days. For waxy starches, the rapid increment occurred in the later stage. According to NMR and DSC analyses, it is apparent that the changes of ΔH and ΔR_2 were similar for indica starches in the first six days storage (Fig. 1a, 5a) and for waxy starches in the first 24 days storage (Fig. 1c, 5b). The equations of ΔH (Table II) and ΔR_2 (Table V) versus time also indicated that the increase of both ΔH and ΔR_2 during storage can be expressed by the same model with different coefficients. This result suggests that the decrease of water mobility may be due to the reassociation of starch molecules.

Leung et al (1983) and Wynne-Jones and Blanshard (1986) indicated that the decrease of water mobility in bread or starch gel

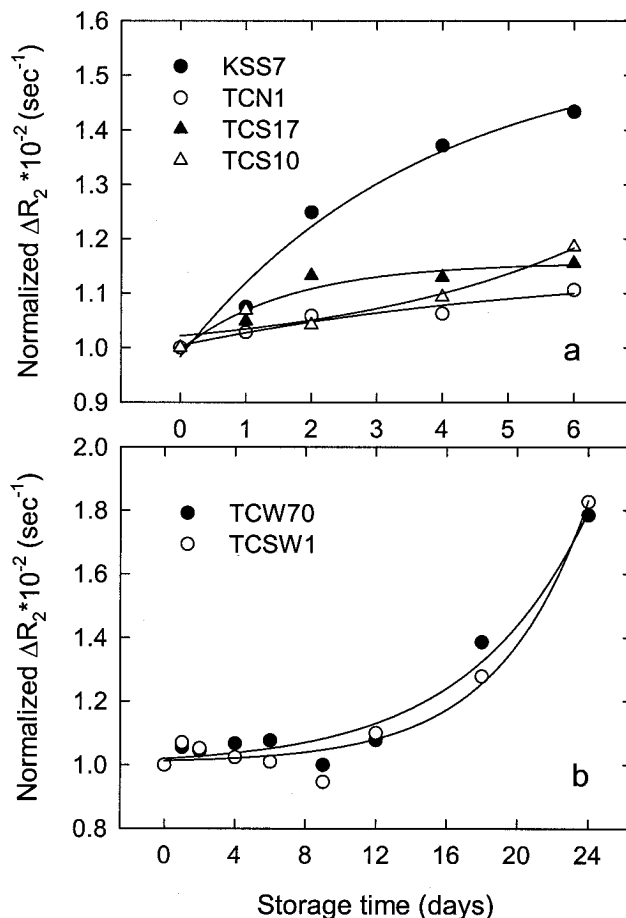


Fig. 5. Nonlinear regression of the normalized differential transverse relaxation rate for ¹⁷O nuclear magnetic resonance (NMR) of water molecules in 25% rice starch cooks with storage time (a, indica; b, waxy).

TABLE III
Freezable Water Content (w/w) of 25% Rice Starch Cooks Measured by Differential Scanning Calorimetry (DSC)^a

Variety	Storage Period (days)				
	0	2	6	12	24
KSS7	64.08 ± 0.10a ^b (6.41 ± 0.21)a	63.28 ± 0.20b (5.58 ± 0.10)b	62.90 ± 0.02b (5.66 ± 0.10)b	61.90 ± 0.55c (5.58 ± 0.20)b	62.29 ± 0.10c (5.17 ± 0.17)c
TCN1	63.36 ± 0.11a (6.27 ± 0.32)ab	62.84 ± 0.23b (6.38 ± 0.20)a	62.55 ± 0.23bc (5.87 ± 0.24)b	62.48 ± 0.10bc (5.92 ± 0.13)ab	62.24 ± 0.29c (5.89 ± 0.21)b
TCS17	63.84 ± 0.77a (6.45 ± 0.55)a	62.55 ± 0.53b (6.03 ± 0.60)a	62.34 ± 0.42b (6.04 ± 0.50)a	62.55 ± 0.07b (6.02 ± 0.02)a	62.23 ± 0.09b (5.84 ± 0.28)a
TCS10	63.63 ± 0.06a (6.26 ± 0.29)a	63.27 ± 0.04b (6.41 ± 0.28)a	62.93 ± 0.12c (6.22 ± 0.09)ab	63.17 ± 0.30bc (5.74 ± 0.20)c	62.89 ± 0.13c (5.83 ± 0.25)bc
TC189	63.26 ± 0.09a (6.29 ± 0.24)a	63.25 ± 0.01a (6.17 ± 0.11)a	63.28 ± 1.11a (6.10 ± 0.16)a	63.02 ± 0.06a (5.79 ± 0.14)b	63.19 ± 0.35a (5.62 ± 0.11)b
TG9	63.26 ± 0.66a (6.35 ± 0.10)a	63.34 ± 0.22a (6.31 ± 0.22)a	63.00 ± 0.21a (6.20 ± 0.26)ab	62.83 ± 0.34a (5.86 ± 0.25)bc	62.84 ± 0.16a (5.79 ± 0.11)c
TNu67	64.15 ± 0.01a (6.46 ± 0.11)a	62.88 ± 0.22b (6.12 ± 0.30)b	62.51 ± 0.13c (6.06 ± 0.04)b	62.63 ± 0.07bc (5.87 ± 0.18)bc	62.77 ± 0.26bc (5.69 ± 0.05)c
TCW70	63.73 ± 0.21a (6.79 ± 0.12)a	63.04 ± 0.24b (6.62 ± 0.26)a	63.02 ± 0.25b (6.63 ± 0.23)a	62.99 ± 0.24b (6.17 ± 0.17)b	63.76 ± 0.31a (5.88 ± 0.24)b
TCSW1	63.56 ± 0.56a (6.61 ± 0.01)ab	63.25 ± 0.13ab (6.74 ± 0.28)a	63.29 ± 0.16ab (6.30 ± 0.21)b	63.22 ± 0.19ab (6.44 ± 0.16)ab	62.73 ± 0.26b (5.64 ± 0.05)c

^a Parentheses represent water fraction melted below subzero temperature.

^b Values followed by the same letter in the same row are not significantly different ($P < 0.05$).

TABLE IV
Water Content in 25% Rice Starch Cooks Measured by Karl-Fischer Method

Days	Water Content (w/w)		
	KSS7	TC189	TCW70
0	68.01 ± 0.80d ^a	60.93 ± 0.69e	61.60 ± 0.18d
2	70.00 ± 0.97c	66.20 ± 1.03d	59.20 ± 0.71e
6	70.67 ± 1.03c	67.38 ± 1.15cd	63.14 ± 0.42c
12	73.70 ± 0.85b	71.69 ± 1.26a	69.03 ± 0.94ab
24	75.51 ± 0.49a	70.37 ± 0.97ab	68.16 ± 0.14b
30	74.11 ± 0.57b	68.75 ± 0.41bc	69.44 ± 0.60a

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

during storage is due to the binding of water to the amylopectin fraction. But Kim-Shin et al (1991) suggested that the redistribution of water during bread staling was most likely related to the water in the amorphous region. The contradictory result may come from overlooking the amylose-amylopectin association and the interaction between bread ingredients such as gluten and water. Hence, we concluded that the water mobility change of starch cook results from amylopectin recrystallization and is promoted by amylose in the initial stage. However, because there was no correlation between amylose content and the initial rate or the exponent of the fitted equation, the exact influence of amylose on water mobility remains unclear. For japonica varieties, the ΔR_2 change did not follow either regression model in the first six days because of the slower recrystallization rate.

CONCLUSIONS

The retrogradation enthalpy changes (ΔH) for rice starch cooks during storage can be classified into three different profiles according to amylose content. The increased bound water determined by DSC corresponded to the incorporation of water into crystalline or network structure and became less mobile. On the other hand, starch chain association weakened the starch-water interaction, which made water more easily extracted by the Karl-Fischer reagent and more mobile. The overall water mobility measured by ¹⁷O-NMR could be governed by both contributions, so the change of differential relaxation rates (ΔR_2) for water-¹⁷O could be more complicated. However, the change over time for both ΔH and ΔR_2 followed the same nonlinear regression models for certain storage periods, except for japonica. This indicates that the decrease of

TABLE V
Nonlinear Regression Equations for Differential Transverse Relaxation Rate (ΔR_2 /sec) of Water Molecules in Rice Starch Cooks as a Function of Time (t , days)

Variety	Equations ($\Delta R_2 =$)	Initial Rate ^a	r^2
KSS7	$0.983 + 0.572 \times (1 - e^{-0.271t})$ ($t \leq 6$)	0.155	0.977
TCN1	$1.004 + 0.150 \times (1 - e^{-0.171t})$ ($t \leq 6$)	0.026	0.933
TCS17	$0.996 + 0.161 \times (1 - e^{-0.601t})$ ($t \leq 6$)	0.097	0.934
TCS10	$0.980 + 0.041 \times e^{0.265t}$ ($t \leq 6$)	0.011	0.907
TCW70	$0.997 + 0.022 \times e^{0.149t}$ ($t \leq 24$)	0.003	0.969
TCSW1	$1.006 + 0.007 \times e^{0.200t}$ ($t \leq 24$)	0.001	0.973

^a Expressed as $d(\Delta R_2)/dt$ at $t=0$.

water mobility accompanies amylopectin recrystallization and that changes of ΔH and ΔR_2 are accelerated by amylose content. The relationship between water mobility and starch retrogradation has been established but further studies are needed to clarify the degree of influence of retrogradation on water mobility.

ACKNOWLEDGMENTS

We wish to thank the Council of Agriculture, Taiwan, for partial financial support, and the Taichung District Agricultural Improvement Station, Taiwan, for providing experimental materials.

LITERATURE CITED

- Baik, M. Y., Kim, K. J., Cheon, K. C., Ha, Y. C., and Kim, W. S. 1997. Recrystallization kinetics and glass transition of rice starch gel system. *J. Agric. Food Chem.* 45:4242-4248.
- Bushuk, W., and Mehrotra, V. K. 1977. Studies of water binding by differential thermal analysis. II. Dough studies using the melting mode. *Cereal Chem.* 54:320-325.
- Chang, S. M., and Liu, L. C. 1991. Retrogradation of rice starches studied by differential scanning calorimetry and influence of sugars, NaCl and lipids. *J. Food Sci.* 56:564-566, 570.
- Chrastil, J. 1987. Improved calorimetric determination of amylose in starches or flours. *Carbohydr. Res.* 159:154-158.
- Chung, K. H., and Lee, C. M. 1991. Water binding and ingredient dispersion pattern effects on surimi gel texture. *J. Food Sci.* 56:1263-1266.
- Fan, J., and Marks, B. P. 1998. Retrogradation kinetics of rice flours as influenced by cultivar. *Cereal Chem.* 75:153-155.
- Giménez, V., Mantecón, A., and Cádiz, V. 1998. Water absorption and states of water in thermosets from ethylene-vinyl alcohol copolymers and dianhydrides. *Acta Polym.* 49:502-509.

- Gudmundsson, M., and Eliasson, A. C. 1990. Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifiers. *Carbohydr. Polym.* 13:295-315.
- Hoseney, R. C. 1984. Chemical changes in carbohydrates produced by thermal processing. *J. Chem. Educ.* 61:308-312.
- Imberty, A., Buléon, A., Tran, V., and Pérez, S. 1991. Recent advances in knowledge of starch structure. *Starch* 43:375-384.
- Jouppila, K., Kansikas, J., and Roos, Y. H. 1998. Factors affecting crystallization and crystallization kinetics in amorphous corn starch. *Carbohydr. Polym.* 36:143-149.
- Kalichevsky, M. T., Orford, P. D., and Ring, S. G. 1990. The retrogradation and gelation of amylopectins from various botanical sources. *Carbohydr. Res.* 198:49-55.
- Kim-Shin, M. S., Mari, F., Rao, P. A., Stengle, T. R., and Chinachoti, P. 1991. ^{17}O nuclear magnetic resonance studies of water mobility during bread staling. *J. Agric. Food Chem.* 39:1915-1920.
- Klucinec, J. D., and Thompson, D. B. 1999. Amylose and amylopectin interact in retrogradation of dispersed high-amylose starches. *Cereal Chem.* 76:282-291.
- Lai, H. M., Jeng, S. T., and Lii, C. Y. 1998. ^{17}O NMR and DSC for studying quality of taro paste as affected by processing and storage. *Food Sci. Technol.* 31:57-63.
- Leung, H. K., Magnuson, J. A., and Bruinsma, B. L. 1983. Water binding of wheat flour doughs and breads as studied by deuterium relaxation. *J. Food Sci.* 48:95-99.
- Li, S., Dickinson, L. C., and Chinachoti, P. 1998. Mobility of "unfreezable" and "freezable" water in waxy corn starch by ^2H and ^1H NMR. *J. Agric. Food Chem.* 46:62-71.
- Liu, Q., and Thompson, D. B. 1998. Retrogradation of *du wx* and *su2 wx* maize starches after different gelatinization heat treatments. *Cereal Chem.* 75:868-874.
- Longton, J., and LeGrys, G. A. 1981. Differential scanning calorimetry studies on the crystallinity of ageing wheat starch gels. *Starch* 33:410-414.
- Lu, S., Chen, L. N., and Lii, C. Y. 1997. Correlations between the fine structure, physicochemical properties, and retrogradation of amylopectins from Taiwan rice varieties. *Cereal Chem.* 74:34-39.
- Radosta, S., and Schierbaum, F. 1990. Polymer-water interaction of maltodextrins. III. Non-freezable water in maltodextrin solutions and gels. *Starch* 42:142-147.
- Richardson, S. J., Baianu, I. C., and Steinberg, M. P. 1987. Mobility of water in corn starch suspensions determined by nuclear magnetic resonance. *Starch* 39:79-83.
- Ring, S. G., Colonna, P., I'Anson, K. J., Kalichevsky, M. T., Miles, M. J., Morris, V. J., and Orford, P. D. 1987. The gelation and crystallisation of amylopectin. *Carbohydr. Res.* 162:277-293.
- Ruan, R., Almaer, S., Huang, V. T., Perkins, P., Chen, P., and Fulcher, R. G. 1996. Relationship between firming and water mobility in starch-based food systems during storage. *Cereal Chem.* 73:328-332.
- Russell, P. L. 1987. The ageing of gels from starches of different amylose/amylopectin content studied by differential scanning calorimetry. *J. Cereal Sci.* 6:147-158.
- Russell, P. L., and Oliver, G. 1989. The effect of pH and NaCl content on starch gel ageing. A study by differential scanning calorimetry and rheology. *J. Cereal Sci.* 10:123-138.
- Sarko, A., and Wu, H. C. H. 1978. The crystal structures of A-, B- and C-polymorphs of amylose and starch. *Starch* 30:73-78.
- Sasaki, T., and Matsuki, J. 1998. Effect of wheat starch structure on swelling power. *Cereal Chem.* 75:525-529.
- Shi, Y. C., and Seib, P. A. 1992. The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydr. Res.* 227:131-145.
- Slade, L., and Levine, H. 1986. Recent advances in starch retrogradation. Pages 387-430 in: *Industrial Polysaccharides*. S. S. Stivala, V. Crescenzi, and I. C. M. Dea, eds. Gordon and Breach Science: New York.
- Vodovotz, Y., and Chinachoti, P. 1998. Glassy-rubbery transition and recrystallization during aging of wheat starch gels. *J. Agric. Food Chem.* 46:446-453.
- Wynne-Jones, S., and Blanshard, J. M. V. 1986. Hydration studies of wheat starch, amylopectin, amylose gels and bread by proton magnetic resonance. *Carbohydr. Polym.* 6:289-306.
- Yang, C. C., Lai, H. M., and Lii, C. Y. 1984. The modified alkaline steeping method for the isolation of rice starch. *Food Sci. (Chinese)*. 11:158-162.
- Yuan, R. C., and Thompson, D. B. 1998. Rheological and thermal properties of aged starch pastes from three waxy maize genotypes. *Cereal Chem.* 75:117-123.
- Yuan, R. C., Thompson, D. B., and Boyer, C. D. 1993. Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three *wx*-containing genotypes in two inbred lines. *Cereal Chem.* 70:81-89.
- Zeleznak, K. J., and Hoseney, R. C. 1986. The role of water in the retrogradation of wheat starch gels and bread crumb. *Cereal Chem.* 63:407-411.
- Zhang, W., and Jackson, D. S. 1992. Retrogradation behavior of wheat starch gels with differing molecular profiles. *J. Food Sci.* 57:1428-1432.

[Received January 23, 2001. Accepted June 6, 2001.]