

Effect of Hydroxypropylation on Retrogradation and Water Dynamics in Wheat Starch Gels Using ^1H NMR

S. G. Choi¹ and W. L. Kerr^{1,2}

ABSTRACT

Cereal Chem. 80(3):290–296

The water dynamics in gels made from native wheat starch, control (alkali-treated) starch, and hydroxypropylated starch were studied using ^1H NMR relaxometry. Transverse relaxation studies showed that at least two domains of water exist in the starch gel, one with a T_2 of 0.5–8 msec and one with a T_2 at 8–200 msec. For starch gels held at 5°C for up to 15 days, the peak T_2 of both regions decreased with time for gels made from native starch, but not for those made from hydroxypropylated starch. Changes in integrated signal in each region suggests that water migrates out of the lower T_2 domain during retrogradation. Gels made from isolated

amylose had a single, relatively mobile water domain, with T_2 dependent on gel concentration. This fraction did not change during storage at 5°C . Granule-rich gels showed two water domains, one with a T_2 range similar to that for amylose gels, which varied over time and were thermally reversible. During storage, most significant changes occurred in the relatively low T_2 region associated with granule remnants. These studies show that, in addition to changes in starch during retrogradation, water dynamics are also affected by recrystallization and chemical modification of starch molecules.

Recrystallization is associated with changes in the physical properties of starch gels including hardness, water absorptive capacity, opacity, degree of hydrolysis, and soluble starch content, and contributes to defects in quality of starch-containing foods (Hoover 1995; Keetels et al 1996a–c). Based on X-ray diffraction studies, Miles et al (1985) proposed a mechanism in which amylose was responsible for short-term irreversible development of gel structure and crystallinity, while amylopectin was involved in long-term thermoreversible crystallization during storage.

The influence of starch source, starch concentration, storage temperature, salts and sugars, lipids, and chemical modifications on starch retrogradation has been studied (Hoover and Sosulski 1986; Orford et al 1987; Wu and Seib 1990; Biliaderis and Tonogari 1991; Teo and Seow 1992; Morikawa and Nishinari 2000). Less understood is how water interacts with starch in the process of retrogradation. It is commonly believed that the molecular motion of water plays an important role in starch retrogradation, and a few studies have focused on the dynamic state of water in starch-based products. Leung et al (1983) proposed that as starch changes from an amorphous to a more crystalline state, water molecules become immobilized by incorporation into the crystalline structure, resulting in a decrease in water mobility during storage. Whistler and Daniel (1985) made differential scanning calorimetry (DSC) measurements of unfreezable water during storage and suggested that water was expelled from the starch matrix due to reassociation of starch molecules. Based on T_1 or T_2 relaxation studies, pulsed ^1H NMR has been useful for quantifying the relative amounts of water in regions of differing water mobility, or in particular, of water with differing degrees of rotational freedom. From ^1H NMR studies, it has been observed that water mobility and the fractions of water in different environments is highly correlated with the degree of starch retrogradation (Wynne-Jones and Blanshard 1986; Ruan et al 1998; Farhat et al 2000). In addition, Le Botlan and Desbois (1995) and Teo and Seow (1992) have shown that ^1H NMR is useful for determining the degree of starch mobility, based on measurements of the signal intensity from the solid-like component in starch gels.

Chemical modification of starch, as by acetylation, cross-linking, or hydroxypropylation, has been used to improve the functional properties and storage stability of starch-based foods. Among them,

hydroxypropylation has been particularly effective at retarding retrogradation, increasing water holding capacity, and improving freeze-thaw or cold-storage stability (Kim et al 1993; Perera and Hoover 1997). This has been attributed to steric effects imposed by bulky hydroxypropyl groups, which prevent alignment of starch chains leading to aggregation and crystallization. The influence of hydroxypropylation on starch retrogradation has been measured by the amount of water exuded during freezing and thawing, turbidity, X-ray diffraction, and DSC. However, little research has been published on the influence of hydroxypropylation on the changes in water relations during storage.

The objectives of this research were to 1) investigate the effect of starch concentration and degree of hydroxypropylation on the dynamic states of water in wheat starch gels during storage, 2) to compare the rate of such changes with that of retrogradation, and 3) to increase further understanding of how hydroxypropylation retards starch retrogradation.

MATERIALS AND METHODS

Preparation of Hydroxypropylated Wheat Starch

Hydroxypropylation of wheat starch was accomplished as described by Kim et al (1992). Native wheat starch (Sigma Chemical Co., St. Louis, MO) was lyophilized for 48 hr, then kept over phosphorous pentoxide (Sigma) to ensure maximum dryness. Dry sample (200 g) was suspended in 440 mL of distilled water containing 40 g of Na_2SO_4 , then adjusted to pH 11.3 with 1N NaOH (Table I). The mixture was placed in 500-mL screw cap jars and maintained at 35°C in a controlled temperature water bath equipped with a rotary shaker. Aliquots (8, 16, or 25 mL) of propylene oxide (Acros Organics) were added to the jars. The jars were immediately capped and vigorously shaken, then returned to the shaker bath. The reaction proceeded for 24 hr with shaking at 120 rpm, and was terminated by adjusting to pH 5.5 using 1N HCl. The suspension was washed with distilled water and centrifuged several times for 15 min at 3,200 g. The hydroxypropyl content was determined by the

TABLE I
Molar Substitution (MS) of Hydroxypropyl Groups
on Wheat Starch Modified with Propylene Oxide

Starch (g)	Na_2SO_4 (g)	Propylene Oxide (mL)	Hydroxypropyl ($\text{C}_3\text{H}_7\text{O}$) (%)	MS ^a
200	10	0	0	0
200	10	8	2.02	0.05
200	10	16	4.11	0.12
200	10	25	6.22	0.18

^a Molar substitution.

¹ Department of Food Science and Technology, University of Georgia, Athens, GA 30602-7610 USA

² Corresponding author. Phone: +1-706-542-1085. Fax: +1-706-542-1050. E-mail: wkerr@uga.edu.

spectrophotometric method of Johnson (1969). The molar substitution (MS) was defined as moles of substituent per mole of anhydro-glucose unit. Control (alkali-treated) wheat starch was prepared with the same reagents and procedure, but without addition of propylene oxide. This was to ensure that any measured changes were due to hydroxypropylation and not from effects of pH change or heating.

Gel Preparation

To prepare homogeneous gels from highly concentrated starch suspensions, a microwave heating method was used. Starch samples (0.5 g) were weighed into glass tubes (3 cm × 1 cm diameter) and

fitted with screw caps. Distilled water (0.75, 1.0, and 1.5 g, respectively) was added to the tube to make a suspension (40, 33, and 25% starch by weight), and vortexed for 20 sec. The sealed containers were placed on a rotating turntable and heated in the microwave oven (Daewoo, Seoul, South Korea) for 40 sec at 800W. After heating, the sample temperature was $94 \pm 2^\circ\text{C}$. Gels were formed as the samples were allowed to cool to 25°C for 1 hr. Fresh gels were immediately measured by NMR. For storage studies, gels were kept at $5 \pm 2^\circ\text{C}$ in a laboratory refrigerator and removed periodically for NMR analysis. All samples were equilibrated to 25°C for 1 hr before measurement.

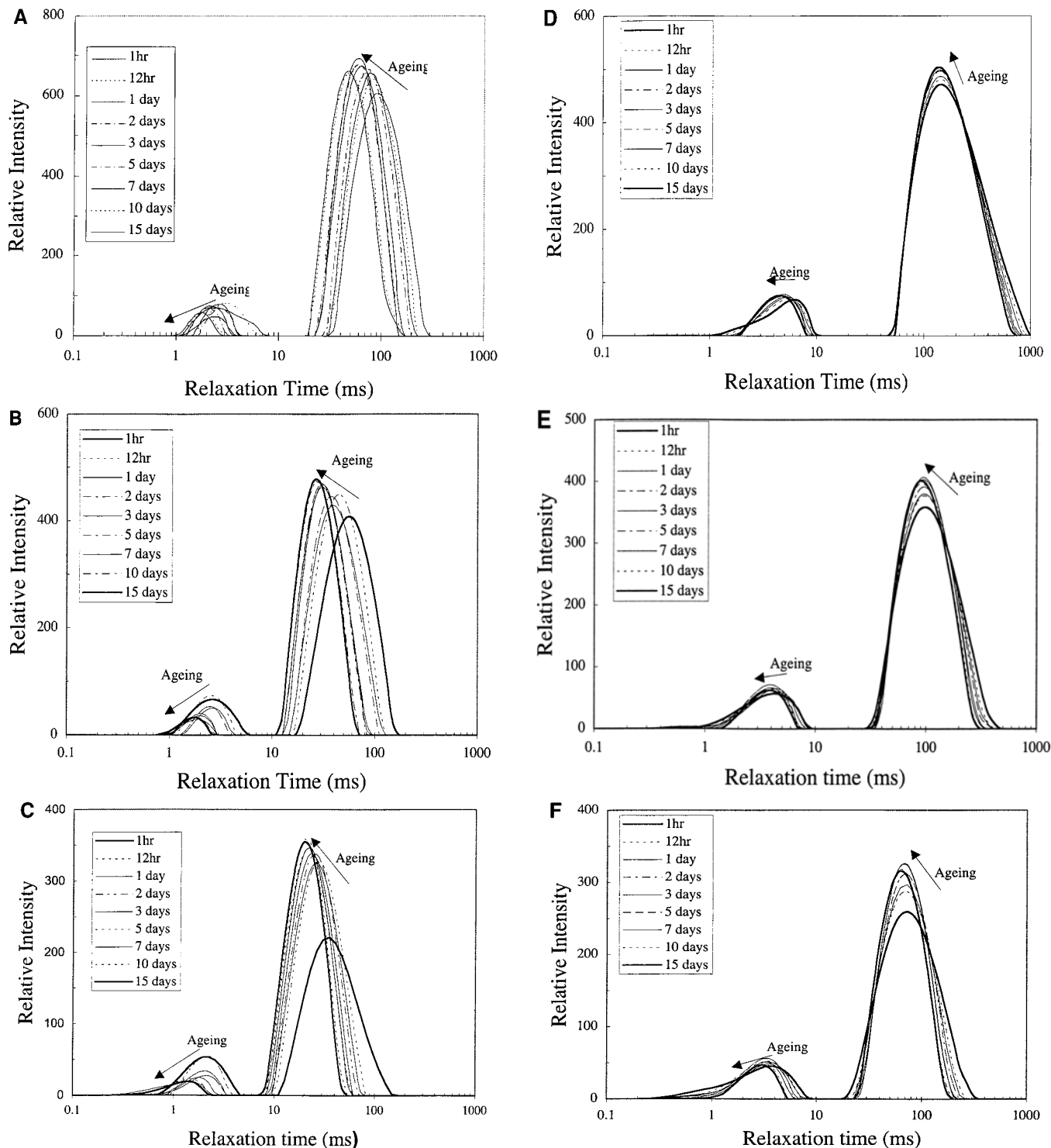


Fig. 1. Distributed exponential fits of T_2 for native wheat starch gels at 25% (A), 33% (B), and 40% (C), and hydroxypropylated (0.18 MS) wheat starch gels at 25% (D), 33% (E), and 40% (F).

Amylose and Granule Remnants

A 7% wheat starch paste was prepared at 70°C and gently stirred for 90 min. The paste was diluted with 200 mL of distilled water at 70°C and centrifuged at 2,500 × *g* for 15 min. The suspension was separated into two phases. The supernant gave a blue color after staining with 0.2% iodine solution (0.2 g of iodine, 2 g of KI, and 100 mL of distilled water) and was clear of granules when examined by light microscopy. This was considered as the soluble starch fraction. The precipitate consisted of gelatinized starch granules when observed at 40×, and gave a purple color when reacted with iodine solution. This was considered to be the insoluble starch fraction. To adjust the concentration of the soluble starch solution, water was allowed to evaporate from the sample while gently stirring at 72°C. The moisture contents of adjusted soluble solution and insoluble starch were determined by drying in vacuo at 75°C for 48 hr. Gels were prepared from the amylose preparations and from the granule-rich sediment as previously described.

NMR Measurement

Proton relaxation measurements were made using a Maran 20-MHz NMR spectrometer (Resonance Instruments, Whitney, UK). The glass tubes with gel specimens were placed in 18-mm diameter NMR tubes, which were covered with parafilm. All measurements were made at 25 ± 0.1°C. Transverse (T_2) relaxation curves were developed using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence: $90x - (t - 180y - \tau - \text{echo})_n$ (Meiboom and Gill 1958). Acquisition parameters were set to a 90° pulse of 4.1 μsec, relaxation delay of 6 sec, and pulse spacing (τ) of 300 μsec. A distributed exponential routine was used to analyze relaxation curves, which calculates a distribution of T_2 terms that best describe the data:

$$g_i = \sum_{j=1}^m f_j e^{-t_i/T_{2,j}} \quad (1)$$

where g_i are the values of the exponential distribution at time t_i , f_j are the preexponential multipliers, and $T_{2,j}$ are the time constants. In general, the distributions assumed more than one distributed region, suggesting the existence of various regions of water with similar relaxation. The number of protons in a given region was measured by the integrated signal intensity over the region of the distribution.

To compare retrogradation behavior over time for native, control, and hydroxypropylated starch at different concentrations, normalized changes in signal intensity were made:

$$\Delta S(t) = \frac{S_0 - S_t}{S_0} \quad (2)$$

where S_0 and S_t are the preexponential factors at time 0 and time t , respectively.

Statistical Analysis

Five different treatment groups were developed: no treatment, control (all reagents and processing except propylene oxide), and three levels of hydroxypropylation (0.05, 0.12, and 0.18 MS). The modification procedure was repeated three times, and three samples were analyzed from each batch. Samples were analyzed over time at 1 and 12 hr, as well as at 1, 2, 3, 5, 7, 10, and 15 days. Analysis of variance (SAS Institute, Cary, NC) was conducted to test for differences among samples at a given time and for differences over time. Nonlinear regression techniques were used to fit the Avrami equation to plots of normalized T_2 versus time.

RESULTS AND DISCUSSION

Hydroxypropylation of Wheat Starch

Analysis of modified starches showed that the molar substitution, defined as the moles of hydroxypropyl per anhydroglucose unit, was 0.05, 0.12, and 0.18, respectively (Table I).

Transverse Relaxation in Starch Gels

The transverse relaxation decay from the CPMG used on starch gels originates from water inside the gels (Farhat et al 2000). A distributed exponential model was used to investigate specific changes during storage of water residing in different regions of the starch gel structure. Figure 1 A–C shows plots of continuous distributions of T_2 for native wheat starch at concentrations of 25, 33, and 40% held at 5°C over 15 days. Two distinct regions of T_2 distributions were observed for all samples, one at relatively short times (≈ 0.5 –8 msec) and one at relatively longer times (8–200 msec). It should be noted that both regions represent relatively short T_2 values in comparison with freely mobile water ($T_2 \approx 2$ sec). Transverse relaxation in gels made from hydroxypropylated starch also showed two populations of water. Initial T_2 values in hydroxypropylated starch gels were greater than that in native starch gels, and samples with greater levels of hydroxypropylation had long T_2 values. Choi and Kerr (2002) attributed this behavior to greater swelling and volume in which water is free to move in hydroxypropylated gels.

Regions of similar T_2 are sometimes related to water mobility in that region, assuming these are due to the effect of fluctuating motions on dipolar interactions. In general, the transverse relaxation rate is related to molecular motion (Hills 1998) by:

$$T_2^{-1} = 0.1A[6J(0, \tau_c) + 10J(\omega_o, \tau_c) + 4J(4\omega_o, \tau_c)] \quad (3a)$$

where the spectral density function is given by:

$$J(n\omega_o, \tau_c) = \frac{\tau_c}{1 + n^2\omega_o^2\tau_c^2} \quad (3b)$$

and A is a constant that incorporates properties of the magnetic field, the spin system, and internuclear distance. $J(\omega)$ measures the number of nuclei with motions at frequency ω and the correlation time τ_c , describes the mean time for motion. Slow motions, particularly those associated with solids, produce rapid relaxation and therefore short T_2 times. Transverse relaxation in protons associated with starch and other biopolymers proceeds too quickly to be seen in the CPMG experiment. Thus, the experiments in this study report on water in the system. In biological systems containing water, tumbling of water molecules is the major type of motion that enhances transverse relaxation (Callaghan 1992).

Other processes, however, may also enhance relaxation. In starch, chemical exchange of water protons with hydroxyl protons occurs, and Tang et al (2001) argued that transverse relaxation in starch largely depends on fast chemical exchange between water and starch protons. This allows for indirect characterization of biopolymer motion, in that faster relaxation associated with less mobile biopolymer leads to faster relaxation in the water. Cross-relaxation between liquid and solid protons may also occur and, in fact, has been used to study relaxation in starch (Wu and Eads 1993; Vodovotz et al 2000).

It is also possible that more than one relaxation rate occurs in a region, even though a single T_2 is observed, as long as fast exchange occurs among elements in the region. The simplest model of this process is the two-site, rapid exchange model:

$$\frac{1}{T_2} = \frac{P_B}{T_{2B}} + \frac{P_A}{T_{2A}} \quad (4)$$

where P_A and P_B are the fractions of, and T_{2A} and T_{2B} are the time constants associated with water in regions with different transverse relaxation rates. For example, if fraction B represents bulk water, and fraction A represents water “bound”, immobilized, or engaging in chemical exchange with a polymer, a single T_2 is observed such that $T_{2A} < T_2 < T_{2B}$.

Transverse Relaxation in Starch Gel Components

The two distributions likely originate from distinct structural regions in the starch gels, or more precisely, populations of water in which diffusion between regions is slow compared to the

relaxation time. Current theories on composite starch gel microstructure suggest that the continuous gel matrix is formed from amylose solubilized from the granule during cooking (Eliasson and Bohlin 1982; Ring and Stainsby 1982). Embedded within this matrix are gelatinized granule remnants composed primarily of branched amylopectin (Hibi 1998; Han et al 2000). Gelation of the amylose-rich network occurs through formation of junction zones, which are crystalline in nature (Zobel and Stephen 1995).

To better identify the peaks, studies on gels made from isolated amylose and granule remnants were conducted. Figure 2A shows distributed fits of T_2 values for amylose gels. In all cases, only a single peak was observed. T_2 values shifted to lower values, and distributions became more narrow, as amylose concentration was increased. The peak T_2 values were 840, 350, and 212 msec, for 1.1, 5.5, and 8.7% gels, respectively. This can be attributed to the increased cross-link density with increasing amylose concentration. This created increased gel rigidity, and in addition, any given water molecule has a shorter distance to diffuse to and interact with neighboring starch chains. Amylose gels did not change in T_2 distributions during storage at 5°C (Fig. 2B). In addition, T_2 distributions did not change when samples stored 10 days were reheated. Although the soluble starch paste was initially clear, it rapidly formed an opaque gel when cooled. This suggests that the amylose gel quickly retrograded, even before NMR measurements could be accomplished. Miles et al (1985) showed that the stiffness of amylose gels did not change during storage over several days.

In contrast, the granule rich sediment (11%) showed two distinct T_2 distributions (Fig. 2C). The larger peak had a maximum T_2 value of 240 msec, while the smaller peak had a maximum T_2 value of 15 msec. The longer T_2 region had values on the same

order of magnitude as that for the amylose gel. This suggests water in a fairly open structure, perhaps in an intergranular gel network. In contrast, the shorter T_2 region is likely due to water in a much more restricted environment. This region is similar to that found in the native starch gel, although at a slightly longer T_2 . This suggests that there was greater swelling in the granule rich sediment than in granules in the full starch gel. It should be noted that the fractionated granule-rich material had more extensive heating than gels made from native starch. Thermal history has been a factor in influencing granule swelling and deformability (Hansen et al 1991; Keetels et al 1996a; Liu et al 1998).

Figure 2D shows that there was a significant decrease in T_2 during aging of the granule-rich material. For example, maximum T_2 values varied from 240, 187, and 100 msec for the long component, and 15, 9, and 6 msec for the short component, at storage times of 0, 1, and 10 days, respectively. Studies on isolated granules have shown that changes in stiffness occur during storage (Miles 1985) and can be traced to an increase in B-type crystal regions. These crystalline regions are thermoreversible and likely involve outermost short branches of amylopectin. Figure 2D shows that T_2 distributions were mostly restored with reheating. The maximum T_2 for the long component was the same as that for the fresh gel. However, there was a broadening of the T_2 distribution.

Changes During Storage of Starch Gels

Figure 1 also shows plots of T_2 distributions for native and hydroxypropylated wheat starch (MS = 0.18) gels at concentrations of 25, 33, and 40%, held at 5°C over 15 days. For native starch gels, peak T_2 values for both water populations decreased with storage time. For hydroxypropylated starch gels, there was no

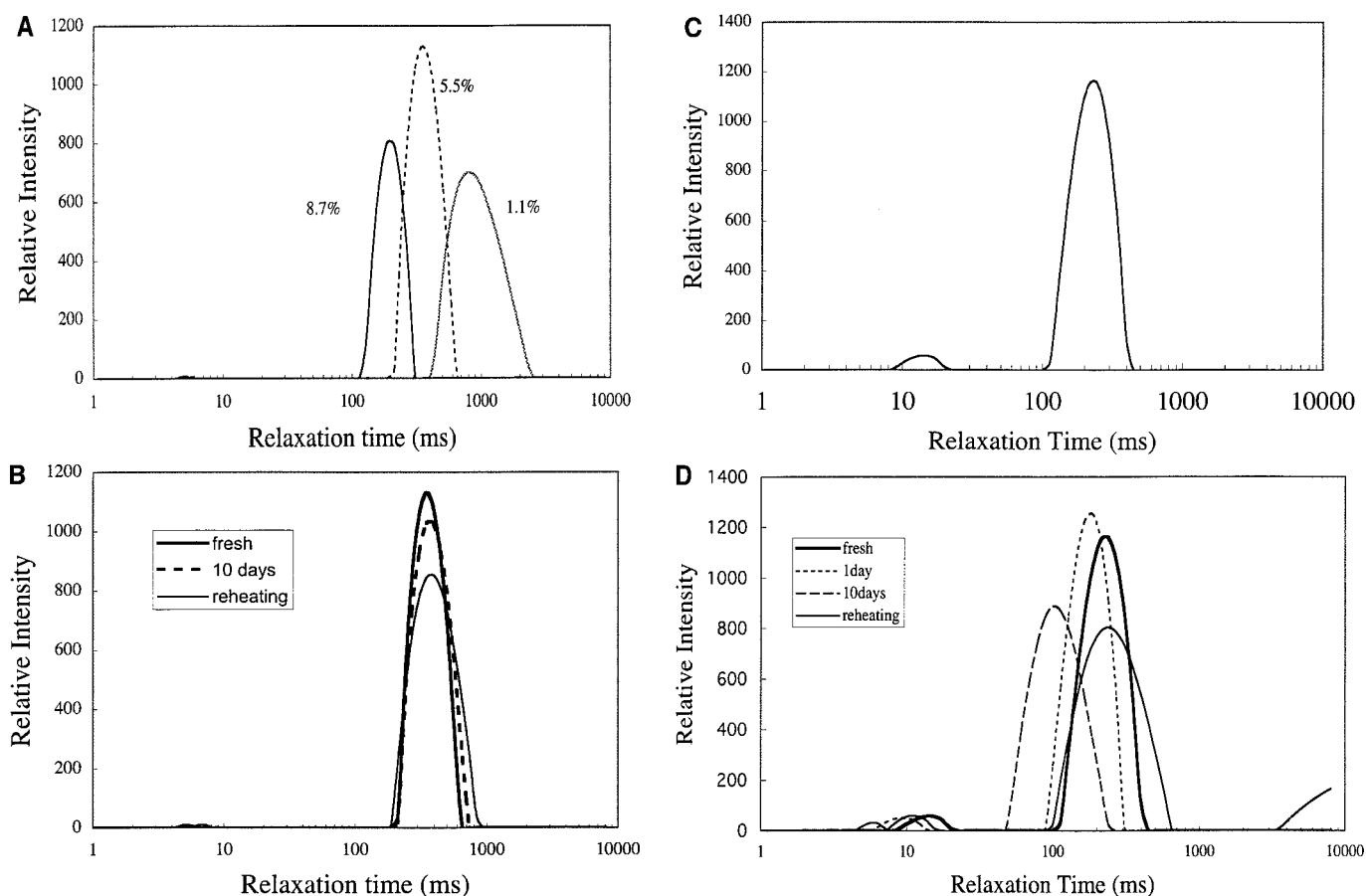


Fig. 2. Distributed exponential fits of T_2 over time for gels made from fractionated starch components. **A**, amylose gels at 1.1, 5.5, 8.7% starch; **B**, 5.5% amylose gels over time and after reheating at 90°C; **C**, granule-rich sediment (11% solids); and **D**, granule-rich sediment during storage and after reheating.

decrease in the average T_2 values for the longer T_2 population. Whether a decrease in T_2 with time indicates lower water mobility is questionable. Probably the most direct effect of aging is on starch chain mobility. Using cross-relaxation NMR techniques, Wu and Eads (1993) showed that there was an increase in relatively immobile starch components. In addition, they used high resolution ^1H NMR to show that there was a decrease in liquid-like mobility. Tang et al (2001) argued that a decrease in starch chain mobility, in turn, creates faster transverse relaxation in the observed water proton populations.

The change in integrated signal intensity during storage for both populations is shown in Fig. 3 A-F, which includes curves for the native, control (alkali-treated), and hydroxypropylated starch gels. Changes in intensity for the more mobile fraction were significant but not large. However, there were no significant differences among

the native, control, or different MS hydroxypropylated starch gels. For the less mobile component, however, changes with storage were more dramatic. The greatest change over time was observed for the native starch. The control starch showed slightly less change in signal intensity, but by days 7 to 15, it was not significantly different than the native starch. Hydroxypropylated starch showed the least change in integrated signal intensity for the less mobile water component. By day 15, the smallest change was observed for the highest MS hydroxypropylated starches at all starch concentrations. The decrease in signal intensity can be attributed to a decrease in water in the less mobile population. This is in agreement with results reported by Ishiguro et al (2000) and Czuchajowska et al (1998), who determined water loss in different starch gel types during storage. Miyazaki et al (2000) suggested that water loss could be used as an index of retrogradation.

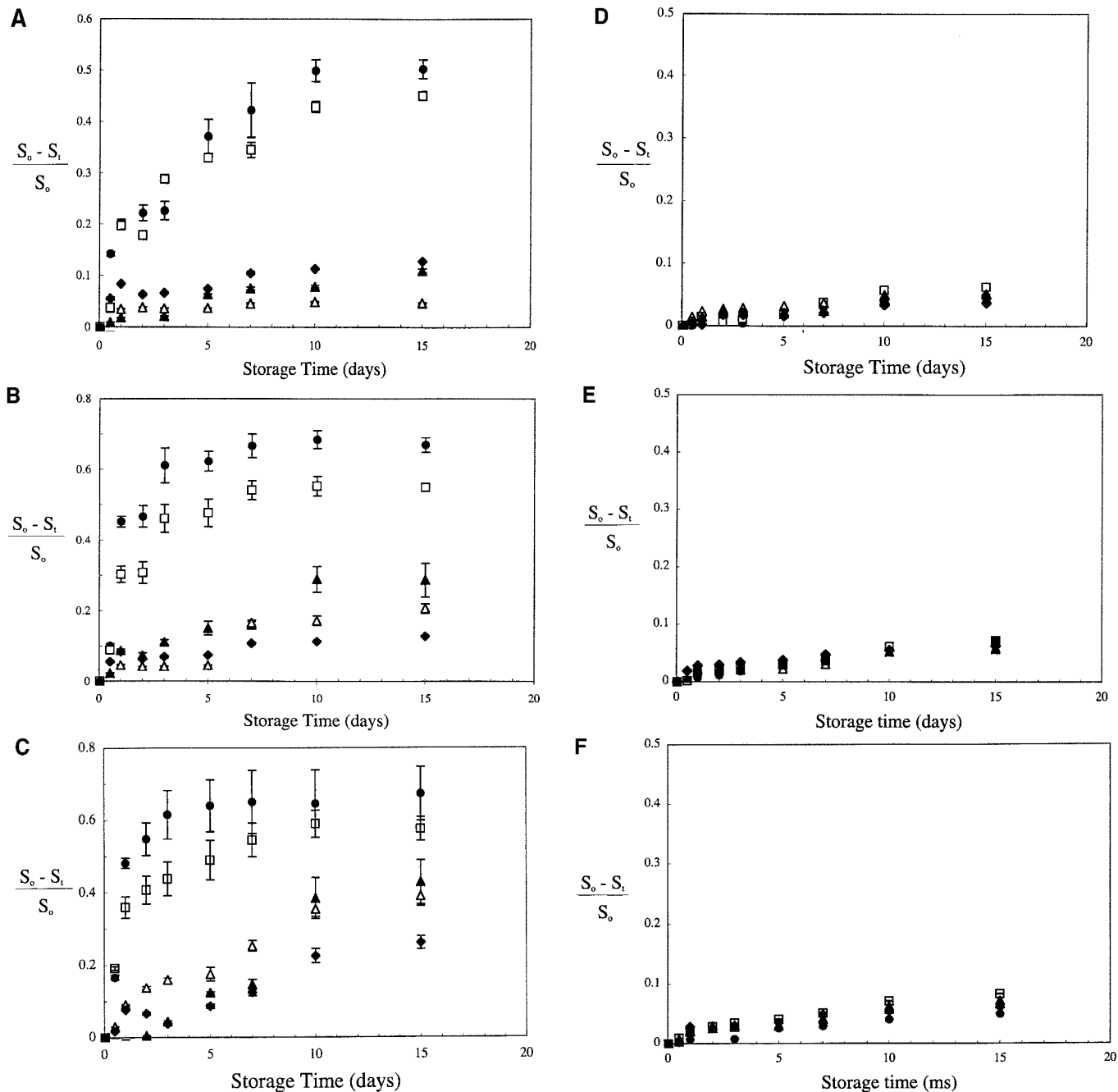


Fig. 3. Normalized signal intensities from more mobile (high T_2) and less mobile (low T_2) regions of native wheat starch gels (●), control (alkali-treated) wheat starch gels (□), and hydroxypropylated starch gels (▲ -0.05 MS, △ -0.12 MS, ◆ -0.18 MS). **A**, less mobile component-25% gel; **B**, less mobile component 33% gel; **C**, less mobile component 40% gel; **D**, more mobile component 25% gel; **E**, more mobile component 33% gel; **F**, more mobile component 40% gel.

These results indicate that changes in the short T_2 fraction of water are most closely associated with retrogradation. Steeneken (1984) reported that at the molecular level, hydroxypropyl groups were mainly distributed in whole amylose molecules and amorphous regions of amylopectin rich in $-1,6$. Substitutions occur in $\approx 33\%$ of the amylopectin backbone, but no hydroxypropyl groups were found in the side chains, which constitute the crystalline regions of amylopectin. Based on X-ray diffraction and birefringence of starch granules, Hoover and Sosulski (1986) reported that all of the hydroxypropyl groups were located in the amorphous regions of the granule. They suggested that the altered structure influences starch retrogradation by decreasing chain aggregation between amylose and parts of amylopectin. As the short T_2 region has been associated with water in granule remnants, the decrease in signal intensity indicates that water is expelled from swollen granule remnants. Similar observations were reported by Morikawa and Nishinari (2000) using a particle size analyzer. They demonstrated that the average particle size of swollen granules decreased as starch retrogradation proceeded.

Retrogradation Kinetics

The Avrami equation has been used to study the kinetics of starch recrystallization in terms of the increase in nucleation and growth of crystallites (Avrami 1940). The fraction of uncrystallized material (θ) at time t is given by:

$$\theta = \exp(-kt^n) \quad (5)$$

where k is the crystal growth rate constant and n is the Avrami exponent that depends on the type of nucleation. In general, the degree of crystallization is determined by X-ray diffraction or differential scanning calorimetry. Below, we have modified Equation 5 to test whether the change in T_2 of the less mobile water fraction is described by Avrami kinetics. Thus:

$$\ln \theta_{T_2} = \ln \left[\frac{T_{2,\infty} - T_{2,t}}{T_{2,\infty} - T_{2,0}} \right] = -kt^n \quad (6)$$

where $T_{2,0}$ is the initial average value, $T_{2,\infty}$ the average value at long storage time, and $T_{2,t}$ the value at time t . Data for all gels was fit to Equation 6 using a nonlinear regression routine. In most cases, the data fit well with $R^2 > 0.92$. Values for k and n were calculated from the fitted curves and are shown in Table II. Values of n for native starch gels ranged from 0.629 to 0.816, depending on starch concentration, which is in good agreement with values reported by Teo and Seow (1992). Values of n for control and hydroxypropylated starches were less (0.324–0.689 for control; 0.271–0.479 for all hydroxypropylated samples), implying that these treatments influence the nature of nucleation during recrystallization. In general, the rate of retrogradation for starch gels as measured by T_2 decreased in the order: untreated, control,

hydroxypropylated (0.05 MS), hydroxypropylated (0.12 MS), and hydroxypropylated (0.18 MS). In addition, as the starch concentration increased, retrogradation proceeded at a faster rate.

CONCLUSIONS

The effects of hydroxypropylation and starch concentration on retrogradation and water mobility in starch gels were investigated. Measurements of T_2 showed that changes occur in the transverse relaxation of water protons during retrogradation. Greatest changes were observed with native wheat starch, less with control (alkali-treated) starch, and even less with hydroxypropylated starch. More detailed studies indicate that at least two domains of water exist in the starch gels. Measurements on isolated amylose gels indicate that a fairly mobile water state is associated with the gel, and which does not change over time and is not thermoreversible. Studies on granule rich fractions show a fairly mobile water state that changes with time, and a more restricted water state that also changes with time. Greatest changes were observed for water in restricted regions of granule remnants.

LITERATURE CITED

- Avrami, M. 1940. Kinetics of phase change. II. Transformation-time relations for random distribution of nuclei. *J. Chem. Phys.* 8:212-224.
- Biliaderis, C. G., and Tonogai, J. R. 1991. Influence of lipids on thermal and mechanical properties of concentrated starch gels. *J. Agric. Food Chem.* 39:833-840.
- Callaghan, P. T. 1991. k-Space microscopy in biology and materials science. Pages 227-318 in: *Principles of Nuclear Magnetic Resonance Microscopy*. Oxford Science Publications: Oxford.
- Choi, S. G., and Kerr, W. L. 2002. Water mobility and textural properties of native and hydroxypropylated wheat starch gels. *Carbohydr. Polym.* 51:1-8.
- Czuchajowska, Z., Otto T., Paszczynska, B., and Baik, B.-K. 1998. Composition, thermal behavior, and gel texture of prime and tailing starches from garbanzo beans and peas. *Cereal Chem.* 75:466-72.
- Eliasson, A. C., and Bohlin, L. 1982. Rheological properties of concentrated wheat starch gels gelatinization, varieties. *Starch* 34:267-271.
- Farhat, I. A., Blanchard, J. M. V, and Mitchell, J. R. 2000. The retrogradation of waxy maize starch extrudates: Effects of storage temperature and water content. *Biopolymers* 53:411-422.
- Han, X., and Hamaker, B. R. 2000. Functional and microstructural aspects of soluble corn starch in pastes and gels. *Starch* 52:76-80.
- Hansen, L. M., Hosene, R. C., and Faubion, J. M. 1991. Oscillatory rheometry of starch-water systems: Effect of starch concentration and temperature. *Cereal Chem.* 68:347-351.
- Hibi, Y. 1998. Roles of water-soluble and water-insoluble carbohydrates in the gelatinization and retrogradation of rice starch. *Starch* 50:474-478.
- Hills, B. 1998. Molecular origins of relaxation contrast. Pages 265-300 in: *Magnetic Resonance Imaging in Food Science*. John Wiley and Sons: New York.
- Hoover, R. 1995. Starch retrogradation. *Food Reviews Int.* 11:331-346.
- Hoover, R., and Sosulski, F. W. 1986. Effect of cross-linking on functional properties of legumes starches. *Starch* 38:149-155.
- Ishiguro, K., Noda, T., Kitahara, K., and Yamakawa, O. 2000. Retrogradation of sweetpotato starch. *Starch* 52:13-17.
- Johnson, D. P. 1969. Spectrophotometric determination of the hydroxypropyl groups in starch ethers. *Anal. Chem.* 41:859-860.
- Keetels, C. J. A. M., Vliet, T. V., and Walstra, P. 1996a. Gelation and retrogradation of concentrated starch system: 1. Gelation. *Food Hydrocoll.* 10:343-353.
- Keetels, C. J. A. M., Vliet, T. V., and Walstra, P. 1996b. Gelation and retrogradation of concentrated starch system: 2. Retrogradation. *Food Hydrocoll.* 10:355-362.
- Keetels, C. J. A. M., Vliet, T. V., and Walstra, P. 1996c. Gelation and retrogradation of concentrated starch system. 3. Effect of concentration and heating temperature. *Food Hydrocoll.* 10:363-368.
- Kim, H. R., Hermansson, A. M., and Eriksson, C. E. 1992. Structural characteristics of hydroxypropyl potato starch granules depending on their molar substitution. *Starch* 44:111-116.
- Kim, H. R., Muhrbeck, P., and Eliasson, A.-C. 1993. Changes in rheological properties of hydroxypropyl potato starch pastes during freeze-thaw treatments. III. Effects of cooking conditions and concentration of the starch paste. *J. Sci. Food Agric.* 61:109-116.

TABLE II

Avrami Constants (Eq. 6) for Starch Gels Held up to 15 Days

Sample	% Starch	k/day	n	R ²
Native	25	0.384	0.629	0.96
	33	0.712	0.644	0.97
	40	0.873	0.816	0.98
Control	25	0.187	0.324	0.98
	33	0.188	0.479	0.98
	40	0.385	0.689	0.95
0.05 MS ^a HP	25	0.067	0.361	0.97
	33	0.098	0.337	0.98
	40	0.150	0.380	0.96
0.11 MS HP	25	0.038	0.372	0.98
	33	0.045	0.461	0.99
	40	0.089	0.461	0.93
0.18 MS HP	25	0.013	0.479	0.99
	33	0.017	0.378	0.92
	40	0.037	0.271	0.76

- Le Botlan, D., and Desbois, P. 1995. Starch retrogradation study in presence of sucrose by low-resolution nuclear magnetic resonance. *Cereal Chem.* 72:191-193.
- 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.
- Liu, H., Ramsden, L., and Corke, H. 1999. Physical properties and enzymatic digestibility of hydroxypropylated ae, wx, and normal maize starch. *Carbohydr. Polym.* 40:175-182.
- Liu, Q., Thompson, D. B., and Donald, B. 1998. Effects of moisture content and different gelatinization heating temperatures on retrogradation of waxy-type maize starches. *Carbohydr. Res.* 314:221-235.
- Meiboom, S., and Gill, D. 1958. Modified spin-echo method for measuring nuclear relaxation times. *Rev. Sci. Instrum.* 29:688-691.
- Miles, M. J., Morris, V. J., Orford, P. D., and Ring, S. G. 1985. The role of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydr. Res.* 135:271-281.
- Miyazaki, K. I., Kumamoto, T. N., Kagoshima, K. K., and Kumamoto, O. Y. 2000. Retrogradation in sweetpotato starch. *Starch* 52:13-17.
- Morikawa, K., and Nishinari, K. 2000. Effects of concentration dependence of retrogradation behaviour of dispersions for native and chemically modified potato starch. *Food Hydrocoll.* 14:395-401.
- Orford, P. D., Ring, S. G., and Carroll, V. 1987. The effect of concentration and botanical source on the gelation and retrogradation of starch. *J. Sci. Food Agric.* 39:169-177.
- Perera, C., and Hoover, R. 1999. Influence of hydroxypropylation on retrogradation properties of native, defatted and heat-moisture treated potato starches. *Food Chem.* 64:361-375.
- Perera, C., Hoover, R., and Martin, A. M. 1997. The effect of hydroxypropylation on the structure and physicochemical properties of native, defatted and heat-moisture treated potato starches. *Food Res. Int.* 30:235-247.
- Ring, S. G., and Stainsby, P. 1982. Filler reinforcement of gels food science. *Progr. Food Nutr. Sci.* 6:323-329.
- Ruan, R. R., and Chen, P. L. 1998. Mobility of water in food and biological systems. Pages 149-228 in: *Water in Foods and Biological Materials, A Nuclear Magnetic Resonance Approach*. Technomic Publishing: Lancaster, PA.
- Steeneken, P. A. M. 1984. Reactivity of amylose and amylopectin in potato starch. *Starch* 36:13-18.
- Tang, H. R., Brun, A., and Hills, B. 2001. A proton NMR relaxation study of the gelatinization and acid hydrolysis of native potato starch. *Carbohydr. Polym.* 46:7-18.
- Teo, C. H., and Seow, C. C. 1992. A pulsed NMR method for the study of starch retrogradation. *Starch* 44:288-292.
- Vodovotz, Y., Dickinson, L. C., and Chinachoti, P. 2000. Molecular characterization around a glassy transition of starch using ¹H cross-relaxation nuclear magnetic resonance. *J. Agric. Chem.* 48:4948-4954.
- Whistler, R. L., and Daniel, J. R. 1985. Carbohydrates. Pages 69-137 in: *Food Chem.* O. R. Fennema, ed. Marcel Dekker: New York.
- Wu, J. Y., and Eads, T. M. 1993. Evolution of polymer mobility during ageing of gelatinized waxy maize starch: A magnetization transfer H NMR study. *Carbohydr. Polym.* 20:51-60.
- Wu, Y., and Seib, P. A. 1990. Acetylated and hydroxypropylated distarch phosphates from waxy barley: paste properties and freeze-thaw stability. *Cereal Chem.* 67:202-208.
- 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.
- Zobel, H. F., and Stephen, A. M. 1995. Starch: Structure, analysis, and application. Pages 19-66 in: *Food Polysaccharides and Their Applications*. A. M. Stephen, ed. Marcel Dekker: New York.

[Received May 29, 2002. Accepted November 17, 2002.]