Proton Relaxation of Starch and Gluten by Solid-State Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT

Proton rotating frame relaxation times [$T_{1\rho}$(H)] were used to characterize the molecular dynamics and structural homogeneity in waxy corn starch, wheat gluten, and mixtures of both. Single-phase relaxation of $T_{1\rho}$(H) was found in native starch, indicating a relatively small dimension of structural heterogeneity in terms of spin-diffusion. Heating of the starch samples decreased the $T_{1\rho}$(H) to 3.2–3.4 msec, as compared to raw starch samples at 5.3–6.2 msec, possibly due to the presence of more amorphous domains. The native wheat gluten displayed a slightly inhomogeneous $T_{1\rho}$(H) of 4.9-6.3 msec, suggesting the presence of a structural inhomogeneity, different from that of native waxy corn starch. Heating of gluten decreased the $T_{1\rho}$(H), which was also dependent on moisture. When mixed at a 1:1 starch-to-gluten ratio and heated, the $T_{1\rho}$(H) associated with the gluten were similar to those for pure gluten at 20% moisture content (mc). However, when dried to 2% mc, the gluten $T_{1\rho}$(H) increased to 9.3–9.6 msec. The $T_{1\rho}$(H) values for starch in the mixture were slightly increased to 5.7 msec. The different $T_{1\rho}$(H) values for starch and gluten suggested a limited miscibility of the two components. Compared to starch, gluten $T_{1\rho}$(H) was far more sensitive to moisture content.

Water, starch and protein are important constituents in foodstuffs. In many aspects, the quality and properties of food products are determined by the molecular dynamics and interactions in the systems. For instance, the retrogradation and crystallization of starch molecules during storage depends, to a large extent, on molecular mobility. This was previously determined by the glass transition temperature (T_g) (Levine and Slade 1988, Kalichevsky and Blanshard 1992). The mobilities and thermodynamic properties of starch and gluten in foods, on the other hand, depend on the moisture content in the system. Water serves as a plasticizer for starch and gluten molecules to lower the glass transition temperature in the food complex (Slade and Levine 1984).

The inherent effects of water on starch and gluten are complicated. If water molecules form hydrogen bonds with starch and gluten molecules, the water relations in food may be interpreted in terms of such interactions. "Unfreezable" water refers to the fraction of water in starch that does not crystallize, even at temperatures far below the freezing point of pure water, within reasonable experimental timeframes (Hally and Snaith 1968, 1971; Kuntz and Kauzmann 1974; Cooke and Kuntz 1974). Many properties of food polymers are related to the unfreezable water. However, the molecular dynamics of starch and proteins in the presence of unfreezable water is unclear. Techniques such as nuclear magnetic resonance (NMR) spectroscopy could provide such information.

Unlike small molecules or synthetic polymers, characterization of molecular dynamics in starch and gluten systems is difficult due to the complexity of the molecular structures. Solid-state NMR has been used to characterize the structure and dynamics of such systems (Veregin et al 1986, Belton et al 1987, Blanshard et al 1990, Grad and Bryant 1990, Hills 1991, Morgan et al 1992, Wu and Eads 1993). With the use of cross-polarization and magic angle spinning (CP-MAS), high-resolution spectra can be readily obtained. The molecular mobilities in the scale of milliseconds to seconds can be characterized using spin-relaxation of both protons and carbons in the laboratory frame and rotating frame (Schaefer et al 1977).

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In this study, we examined the proton relaxation in the rotating frame for waxy corn starch and wheat gluten. The effects of water were studied at various moisture contents for both the starch and gluten samples. Effects of heating (gelatinization for starch and denaturation for gluten) were also investigated for starch and gluten samples and for samples mixed 1:1.

MATERIALS AND METHODS

Materials

Waxy corn starch samples (Waxy No. 1, A.E. Staley, Decatur, IL) had a 10.5% mc (total basis), as measured by thermogravimetric analysis (TGA). Native wheat gluten samples (G-5004, Sigma Chemical, St. Louis, MO) with 80% protein content and 7% fat had a 14.9% mc as measured by TGA. Three kinds of samples were prepared for the NMR measurements: 1) native, 2) heated and 3) gluten heated and cooled stepwise.

Sample Preparation

Native waxy starch (10.5% mc) was used as is. Native starch of 2.0% and native gluten of 2.0 and 7.5% mc were prepared by drying the native samples at 60°C in vacuum oven.

Heated samples were prepared by hydrating starch and gluten samples to ≈50% mc, and then spreading samples evenly onto a flat-bottomed disk covered with a ground-joint lid (to minimize water loss). Samples were heated for 15 min in a preheated oven (150°C). During heating, the sample temperature rose to 100°C in ≈10 min, and remained relatively unchanged thereafter. The heated samples were then cooled, dried in open air at ambient temperature to ≈30–40% mc, and then further dried at 60°C in a vacuum oven. Heated starch at 2.0 and 12.0% mc and heated gluten at 2.0 and 20.0% mc were prepared in this fashion. Typically, it took ≈4 hr to reach a 20% mc and three days to reach a 2% mc. Heated sample mixtures (1:1, starch and gluten) were prepared similarly. Approximately 5 g of waxy corn starch and 5 g of wheat gluten were mixed and hydrated with 10 g of water. The mixture was spread onto a flat-bottomed disk with ground-joint lid and heated as described above. The heated mixtures were then cooled and dried to 2.0 and 20.0% mc.

Samples were stored in sealed containers at ambient temperature. Polarized optical microscopy showed no sign of crystalline structure (lack of maltese cross) of waxy corn starch during storage.

Dried samples were ground into fine powder in liquid nitrogen using a Spex Freezer Mill (Spex Industries, Edison, NJ) for NMR measurements. This practice was necessary to facilitate solid state CP-MAS NMR experiments.

Gluten heated and cooled stepwise samples were prepared to study the effect of temperature and water on the molecular dynamics of gluten. Heated gluten at 2.0 and 20.0% mc (same heating method as above) was cooled from ≈60 to ≈30°C, stepwise, with ≈30 min at each step. Cooling was done in the NMR instrument and $T_{1\rho}$(H) was measured at each temperature.

NMR Analysis

^{13}C spectra, ^{1}H spectra and the $T_{1\rho}$(H) were obtained using a Bruker ASX 300 NMR spectrometer. Samples were packed into 7-mm ceramic rotors and spun at ≈3,500 Hz at MAS to eliminate chemical shift anisotropy. The ^{13}C spectra and $T_{1\rho}$(H) were obtained with CP pulse sequence to enhance the sensitivity.

Figure 1 shows the pulse sequence for the $T_{1\rho}$(H) measurements. The $T_{1\rho}$(H) values were obtained at ambient temperature by spin-locking the proton spins in the rotating frame for a variable delay (spin-lock time) before cross-polarization (Li et al 1994a). The proton spins were flipped to the rotating frame by a 5 μsec 90° pulse and were immediately locked with a spin-lock field strength of 50 kHz. After a variable spin-lock time (τ) in the rotating frame, the proton spins were brought in contact with ^{13}C spins with a fixed contact time of 2 msec for the cross-polarization and followed by acquisition of the ^{13}C signal. Thus, the residual proton magnetization after relaxation in the rotating frame for a delay (τ) was measured using the ^{13}C spins. The details of the experiments were described elsewhere (Li et al 1994a). Typically the $T_{1\rho}$(H) was determined by fitting about 10–12 data points (τ) using a one-component or two-component model. Values were considered valid when the standard error of the fitting was within the order of 10^{-2}. NMR analysis of starch, gluten, and starch-gluten mixtures were performed at ambient temperature. Additionally, some wheat gluten was subjected to measurement at various temperatures from ≈60 to ≈30°C (stepwise cooling done using a Nestlab chiller with air with a –73°C dewpoint).

^{1}H NMR spectra for gluten and starch were also obtained using a 3.5 μsec 90° pulse and a 101,800 Hz spectral width. The large spectral width was necessary to observe both the wideline resonance of the solid protons as well as the narrow resonance of the liquid ones (Wu et al 1992). Some hydrated gluten was spiked with excess water by injecting ≈10 μl of water into 250 mg of dried sample (2.0% mc) immediately before data acquisition. The ^{1}H NMR spectrum collected in this fashion might lead to one resembling a superposition of spectra of gluten protons and free water protons.

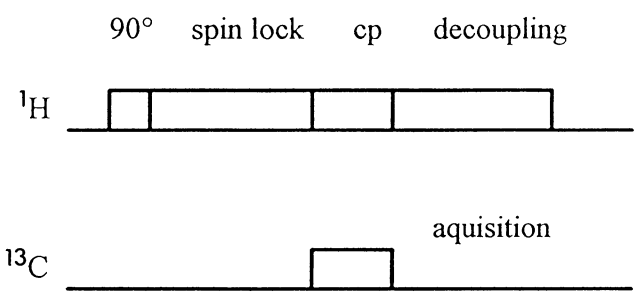

Fig. 1. Pulse sequences used for the measurement of proton rotating frame relaxation times [$T_{1\rho}$ (H)]. Proton magnetization was spin-locked in the rotating frame for various times, and the decay of magnetization was measured using ^{13}C cross-polarization (cp). ^{13}C spectra were obtained with zero spin-locked time.

Moisture Analysis

The water content of the samples was measured by TGA. Samples were heated at a rate of 2°C/min from 30 to 250°C with nitrogen gas purge (Fig. 2). The weight loss due to the evaporation of water was continuously monitored, and the water content of the samples was measured by the weight loss at 180°C. Further heating to a higher temperature was necessary to assure a smooth baseline, but this might have involved thermal decomposition. The weight loss measured by TGA at 180°C was consistent with values obtained from the standard vacuum oven drying method (60°C at 736 mm Hg vacuum for two days). Two weight loss curves (heating rates of 2°C/min and 20°C/min) are shown in Figure 2. The results indicated that the weight loss from water evaporation was independent of heating rate. The water content was determined immediately after the NMR measurements.

RESULTS AND DISCUSSION

CP-MAS ^{13}C Spectra

Native waxy corn starch is a semicrystalline powder that contains both crystalline and amorphous structures. The multiphase structure in the sample can be detected by solid-state NMR. Figure 3A shows the CP-MAS ^{13}C spectra for native (10.5% mc) and heated starch (12.5% mc) samples in comparison with A-type starch α-(1→4)-glucan (Blanshard et al 1990). The resonance at 95~103 ppm represented the C1 carbon, the resonance at 60 ppm corresponded to the C6 carbon, and the strong signals at around 71 ppm represented the resonance from C2–C5 carbons in starch. The amorphous C4 resonance appeared as a weak peak at 79 ppm (Veregin et al 1986).

The crystalline structure in starch has been identified by the line shape in the C1 resonance (Veregin et al 1986). This and other groups (Belton et al 1987, Blanshard et al 1990) characterized a triplet splitting in the C1 carbon resonance due to the A form crystalline structure (Fig. 3A, top spectrum) and a doublet splitting due to the B form crystalline structure of starches.

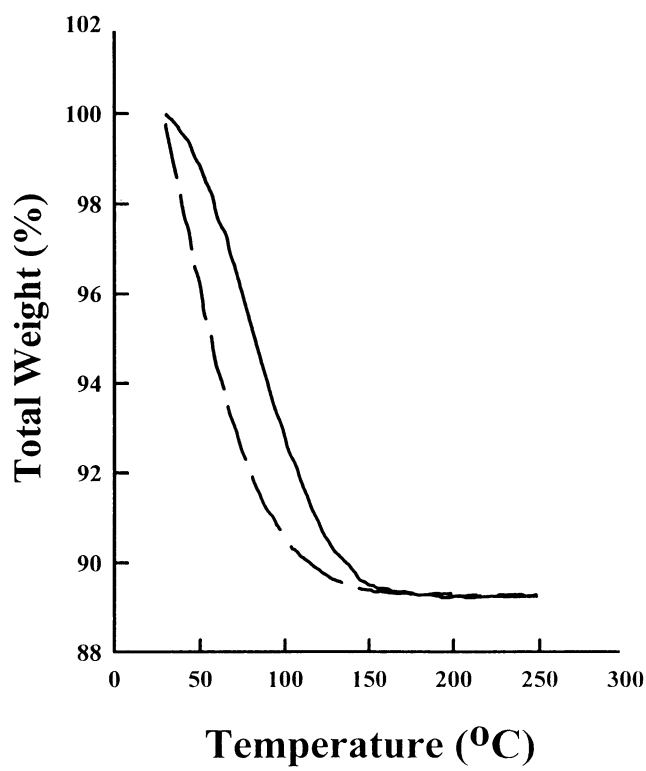

Fig. 2. Thermogravimetric analysis curves for moisture content in native waxy corn starch heated at 2°C/min (– – –) and at 20°C/min (——). Moisture content was measured by the weight loss at 180°C.

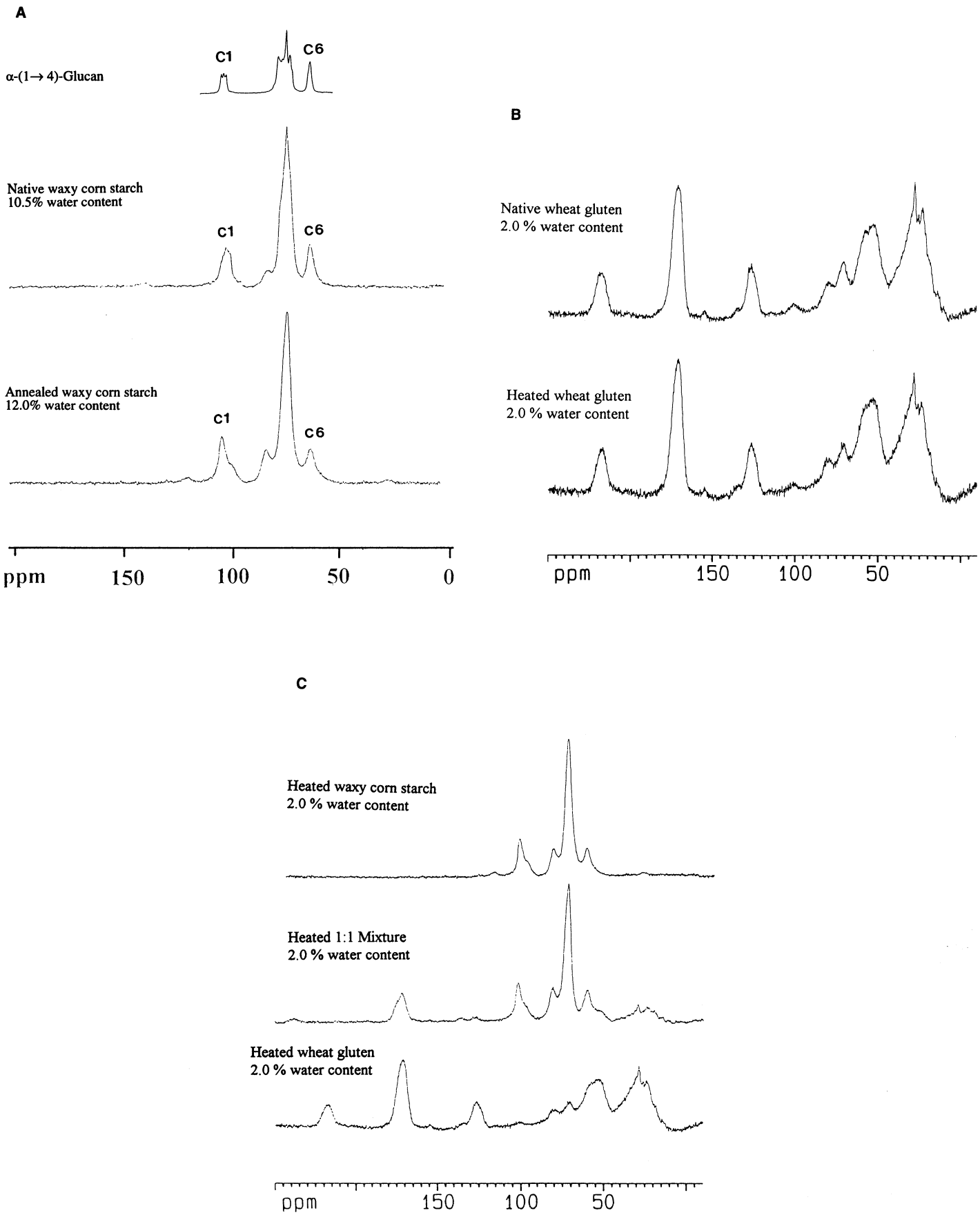

Fig. 3. ^{13}C spectra for A-type α-(1→4)-glucan (Blanshard et al 1990). **A,** Native waxy corn starch with 10.5% water content and heated waxy starch with 12.0% water content. **B,** Native and heated wheat gluten at 2.0% water content. **C,** Heated waxy starch, 1:1 starch and gluten mixture, and heated wheat gluten at 2.0% water content. Spectra were acquired at ambient temperature.

The C2 carbon from the amorphous region was reported to give rise to broad shoulders to the peaks in the 95~103 ppm region (Veregin et al 1986). Figure 3A shows that the C1 resonance in the 95-103 ppm region for native starch is broader than that for the heated sample. This might originate from the weak triplet characteristic of the crystalline structure in the native starch. The lineshape of the spectrum might be affected by a number of factors including starch types used and the presence of a great fraction of amorphous structure in the waxy corn starch samples (100% amylopectin). Similar results have been reported for dried potato starch (Veregin et al 1986). Heating of starch resulted in a narrower C1 peak with no triplet characteristics. The change in the resonance reflected the structural transition from crystalline to amorphous states due to heating. The absence of crystalline structure was also confirmed by a complete loss in birefringence by polarized optical microscopy (data not shown).

Figure 3B shows the CP-MAS ^{13}C spectra for native and heated wheat gluten samples. Because the molecular structure for wheat gluten is extremely complex, detailed assignment of each resonance peak was not possible. But in general, peaks for carbonyl, aromatic, and aliphatic carbons are located at ≈180, ≈135, and ≈35 ppm, respectively (Baianu 1981, Baianu et al 1982, Moonen et al 1985). Heating had no observable impact on the gluten (Fig. 3B). Ablett et al (1988) also reported no significant changes in high resolution ^{13}C NMR intensities after heating and cooling. They suggested that the environments of mobile regions were not altered by heating and that heat setting resulted from other interactions, mainly disulfide bond formation.

Figure 3C shows the CP-MAS ^{13}C spectrum for the heated starch, gluten, and 1:1 starch-gluten mixtures, all at 2.0% mc. In general, peaks associated with starch also appeared in the 1:1 mixture. Similarly, gluten peaks also appeared in the 1:1 mixture spectrum (except for 220 ppm) and, thus, the spectrum for the starch-gluten mixture was basically a superposition of the spectra for starch and gluten.

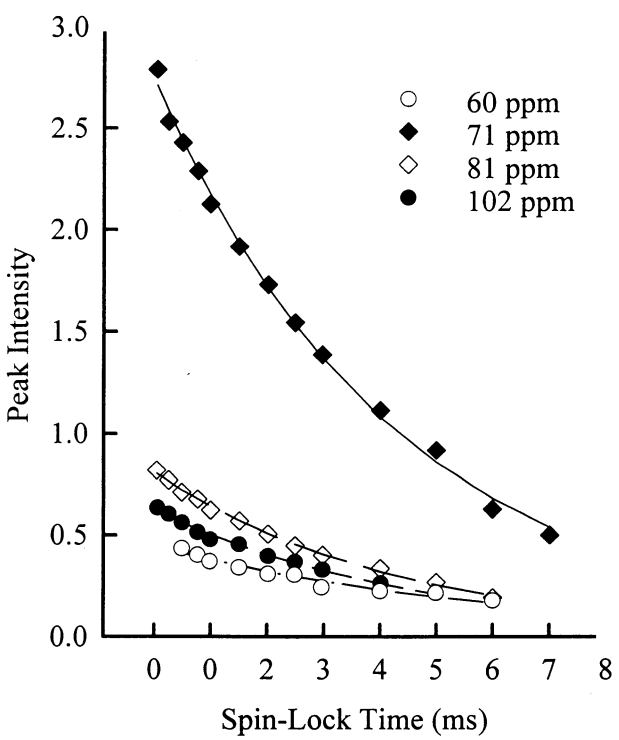

Fig. 4. ^{13}C nuclear magnetic resonance intensities for heated waxy starch at 12.5% water content as a function of spin-lock time at ambient temperature. Lines are the best fits from the one-component model.

$T_{1\rho}$(H) of Waxy Corn Starch

The $T_{1\rho}$(H) values were measured by the spin-lock time dependence of peak intensities at the C1 (102 ppm), C2-C5 (71 ppm), and C6 (60 ppm) resonance for waxy corn starch. Figure 4 shows the typical plot of ^{13}C resonance intensities as a function of spin-lock time (τ) for native waxy starch (10.5% mc) at ambient temperature. No attempt was made to deconvolute the contributions from the crystalline and amorphous regions. Thus, the intensities [$M(t)$] represented the sum of both structures. The intensity of a certain resonance (Li et al 1994a) could be expressed as:

$$M(t) = M_c(0) \exp[-\tau/ T_{1\rho}^c(H)] + M_a(0) \exp [-\tau/ T_{1\rho}^a(H)] \quad (1)$$

where $M_c(0)$ and $M_a(0)$ are the intensities at $\tau = 0$ from the crystalline and amorphous regions, respectively, and $T_{1\rho}^c$(H) and $T_{1\rho}^a$(H) are the corresponding rotating frame relaxation times for the crystalline and amorphous regions, respectively.

However, the measured $T_{1\rho}$(H) value is much more complicated than the two-phase model presented above. As shown in Figure 5, the observed ^{13}C resonance was the result of several simultaneous processes. Proton spins in the solid state, such as in the crystalline and the amorphous regions, are subject to strong dipole-dipole interactions. The dipole-dipole interaction results in a spin-spin relaxation between protons in the two regions. This process is often known as spin-diffusion (Abragam 1961, McBriety and Douglass 1980, Li et al 1994b). In such a system, the observed $T_{1\rho}$(H) is determined by factors such as domain size ($\langle r \rangle$), spin-diffusion rate (D), and the individual $T_{1\rho}$(H) in each domain. If

$$\langle r \rangle \ll \sqrt{D\,(T_{1\rho}(H))^2} \quad (2)$$

then only an average $T_{1\rho}$(H) will be observed:

$$1/ T_{1\rho}(H) = N^c / T_{1\rho}^c(H) + N^a / T_{1\rho}^a(H) \quad (3)$$

where N^c and N^a are the number of protons in the crystalline and amorphous regions, respectively.

Protons from water molecules also participate in the spin-spin relaxation with protons in the polymers through cross-relaxation (Grad and Bryant 1990) and chemical exchange (Schmidt 1990). In either case, the result is the same as spin-diffusion: the observed $T_{1\rho}$(H) tends to be homogeneous. It was possible that some of the protons of water closely associated with the solids (macromolecules) also participated in the cross-polarization.

The NMR data were fitted with a one-component model as shown by the lines in Figure 4. The one-component model fitted well with the experimental data. A two-component model was also attempted, but it failed to improve the degree of fit. The resulting $T_{1\rho}$(H) values for the starch samples under various conditions are given in Table I.

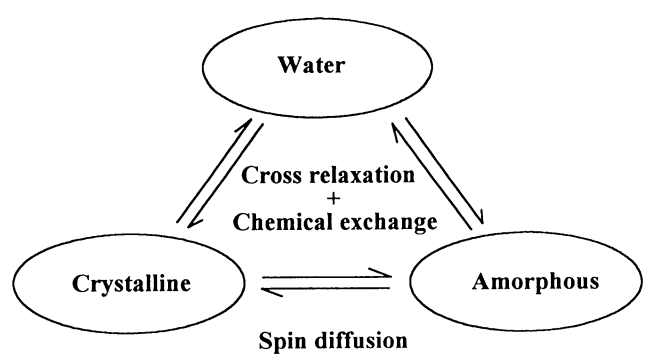

Fig. 5. Possible mechanisms of spin-spin interactions in a food polymer system.

Table I shows that, for waxy corn starch, all protons exhibited a fairly uniform relaxation time in the rotating frame (from 5.3 msec for the C2–C5 signals to 6.2 msec for the C1 signals). The values generally fell between those of amorphous and crystalline amylopectin reported by Morgan et al (1992). Because the signals represented the contributions of protons from both the amorphous and the crystalline regions, the measured $T_{1\rho}(H)$ values were the average of the two phases.

There are several possible mechanisms for the observed single relaxation in the system. As discussed earlier, cross-relaxation (between the mobile and rigid protons) as well as the chemical exchange are known to reduce the heterogeneity in relaxation rate among different protons (Grad and Bryant 1990). However, these mechanisms usually occur in the time scale of $T_1(H)$, and thus are negligible in the $T_{1\rho}(H)$ scale. The proton spins in solids are also homogenized through spin-diffusion. The rate of homogenization of various proton relaxation times depends on the distribution of protons in the system. If all protons are well mixed, a single relaxation time could be observed. However, if the protons distribute heterogeneously beyond the spin-diffusion range, multiple relaxation times would be observed. For synthetic polymers, to detect the heterogeneity in relaxations, a minimum of dimension of 1 nm in each region is required (McBriety and Douglass 1980, Li et al 1994b). The uniform relaxation time obtained from different carbon resonance in Figure 4 would mean that the size of the heterogeneity domains was <1 nm.

To observe multiple relaxation, the crystalline and amorphous domains must be greatly different. It has been reported that $T_{1\rho}(H)$ values for wheat starch were 4 and 8 msec for the amorphous and crystalline regions, respectively (Morgan et al 1992). This was a small difference, and it might have caused some difficulty in distinguishing the relaxations in the crystalline and amorphous domains in the native waxy corn starch in this study.

$T_{1\rho}(H)$ for the heated starch samples with 2.0 and 12.0% mc are also shown in Table I. In general, the heated starch samples showed lower $T_{1\rho}(H)$ values (3.6 msec for C1, 3.2 msec for C2~C5, and 2.1 msec for C6) than that of native starch samples at similar moisture content (12.0% mc heated starch vs. 10.5% mc native starch). These $T_{1\rho}(H)$ values were consistent with the values for amorphous starch ($T_{1\rho}(H)$ = 4 msec) (Morgan et al 1992). Comparing 2.0% and 10.5% mc (Table I), moisture content did not seem to have significant effect on the $T_{1\rho}(H)$ of the heated starch. This insensitivity of $T_{1\rho}(H)$ to moisture for the heated starch samples is not clearly understood. Within this moisture range, starch reportedly shows a glass transition temperature range of 25–70°C (i.e., 20°C sample is in a glassy state) (Kalichevsky and Blanshard 1992, Cherian and Chinachoti 1996). It is possible that, because starch was glassy at the 2–12% mc range, mobility of starch molecules did not change significantly with changing moisture content.

TABLE I
Proton Rotating Frame Relaxation Time [$T_{1\rho}(H)$] of Waxy Corn Starch at Different Moisture Contents (mc) and Heat Treatments

		$T_{1\rho}(H)$ (msec)		
Temperature	mc (%)	C1 (102 ppm)	C2–C5 (71 ppm)	C6 (60 ppm)
Unheated	10.5	6.2	5.3	5.9
Heated at 100°C/10 min	2.0	3.3	3.2	3.4
Heated at 100°C/10 min	12.0	3.6	3.2	2.1

TABLE II
Proton Rotating Frame Relaxation Time [$T_{1\rho}(H)$] of Wheat Gluten at Different Moisture Contents (mc) and Heat Treatments

		$T_{1\rho}(H)$ (msec)			
Temperature	mc (%)	171 ppm	71 ppm	52 ppm	29 ppm
Unheated	2.0	5.4	4.9	6.3	5.2
Unheated	7.5	4.3	3.1	4.4	4.5
Heated at 100°C/10 min	2.0	4.3	4.3	4.8	4.9
Heated at 100°C/10 min	20.0	3.3	3.5	3.9	3.6

$T_{1\rho}(H)$ of Gluten

$T_{1\rho}(H)$ values were measured for the native (2.0 and 7.5% mc) and heated gluten (2.0 and 20.0% mc) samples. The $T_{1\rho}(H)$ values are listed in Table II for selected resonance (171, 72, 52, and 29 ppm). As can be seen from Table II, native wheat gluten showed a slight difference in $T_{1\rho}(H)$ for protons associated with different carbons (4.9 msec at 72 ppm, 6.3 msec at 52 ppm, 5.2 msec at 29 ppm). The relaxation data also showed some degree of multiple relaxation or decays (data not shown), indicating a heterogeneity of the gluten structure. Similar to waxy corn starch, heating and drying to a given moisture content resulted in a lower and more uniform $T_{1\rho}(H)$ among protons associated with different carbons (compare the values for samples of 2.0% mc). This was possibly because of a homogeneous and more mobile structure of heated wheat gluten. Increasing moisture content also decreased the $T_{1\rho}(H)$ for both the native and heated gluten samples studied. Thus, the effect of heating should be compared for samples of same moisture content.

It is also seen from Table II that the $T_{1\rho}(H)$ of wheat gluten was dependent on moisture content. Increasing the moisture content in both native and heated gluten resulted in a decrease in $T_{1\rho}(H)$. This dependence on water (as opposed to relative independence of waxy corn starch on water) suggested a mechanism of water-gluten interaction distinct from that of water-starch interaction. It is speculated that wheat gluten has a stronger affinity for water molecules and, at a lower moisture content, the water in gluten sample was in a relatively immobile phase and in close vicinity to the protein molecules. It is suggested that the measured $T_{1\rho}(H)$ of gluten may also include some contribution of the water protons.

This speculation is contrary to suggestions by others who proposed that starch had a stronger affinity for water than did gluten (Bushuk and Winkler 1957). Interaction between water and macromolecules should affect the molecular mobility of water molecules at the water-solid interface. Bushuk and Winkler (1957) reported that water is "bound" more to starch than to gluten, based on its higher water sorption capacity. However, the amount of water sorbed cannot reveal the molecular mobility and the degree of interaction. Li et al (*in press*) reported the water sorption isotherm of a starch could be described based on a solution-gel model that takes into account the physical trapping of water in starch gel. Thus, the amount of water sorbed can be also caused by the physical and physicochemical entrapment of the polymers and not necessarily by chemical interaction.

This speculation was also supported by the results from wide-line ^1H NMR of heated wheat gluten as shown in Figure 6. The spectra for both the 2.0 and 7.5% mc samples, which showed only mobile proton signals, appear to be almost identical. Resonance for water protons (normally at ≈5.4 ppm) was broad and became part of the baseline. This result suggested that for this amount of water in gluten, the water was associated with the gluten molecules in the solid phase and experienced very low mobility. Thus, the water protons could not be seen in such normal proton spectra. When spiked with liquid water (Fig. 6), it was clear that free-water peak appeared distinctively, while our unspiked samples did not show such peaks.

In contrast to gluten, starch samples (Fig. 6) showed no narrow ^1H peak at 2.0% mc, but a strong mobile proton peak at 10.5% mc, indicating a presence of mobile fraction due to uninteracted water at 10.5% mc. It is speculated that water protons were more mobile in starch and not closely associated with starch molecules at 10.5% mc. These data also suggested that water in starch was mobile while water in gluten remained immobile, at least up to 7.5% mc. This is supported by Umbach et al (1992), who con-

cluded that the small amount of water in dry starch was very tightly associated, but additional water did not interact with starch and thus remained quite mobile. They also reported that, once further hydrated, water in gluten (although reported to have some mobility) led to more water-protein interaction. Our data also supported their conclusion that proteins are more rotationally mobile than starch (Fig. 6): gluten at 2.0% mc showed many narrow peaks while starch did not, but these protons are likely to be protein protons. Umbach et al (1992) reported self-diffusion coefficient of mobile protons in dry gluten samples, suggesting that these may have a significant contribution from both water and proteins. Without a way to distinguish the contributions from water versus proteins, it is impossible to draw any conclusions on water self-diffusion coefficient in such samples.

Cherian and Chinachoti (1996) studied ^2H NMR of hydrated gluten and reported a very weak (almost none) water signal detected at <10% mc, which corresponded to a change in state from rubbery to glassy state (from thermal analysis). They also suggested that the lost signal (decreased in mobile deuterons) could come from decreased mobility of water or the protein side chains, or both.

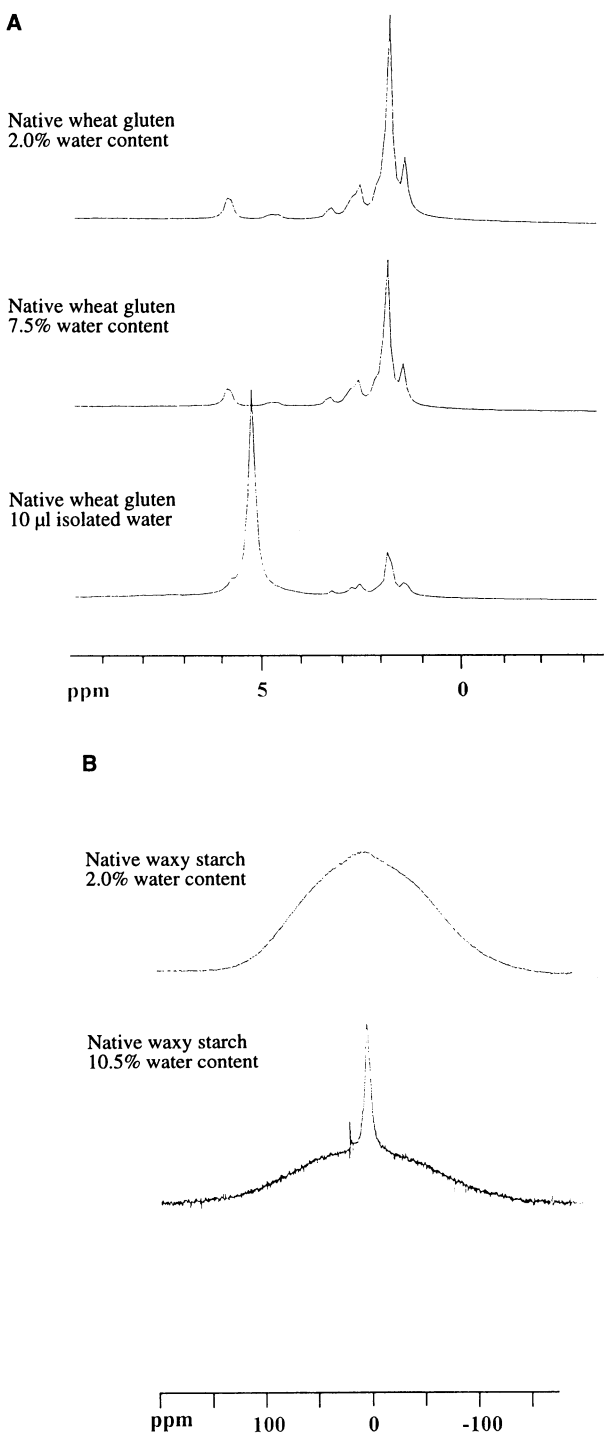

Fig. 6. Wideline ^1H nuclear magnetic resonance of native wheat gluten **(A)** and native waxy starch **(B)** at ambient temperature.

$T_{1\rho}$(H) of 1:1 Waxy Corn Starch and Wheat Gluten Mixture

Table III lists the $T_{1\rho}$(H) for the heated 1:1 mixture of waxy starch and wheat gluten, along with the values for the heated individual components for comparison. For the heated mixture with 2.0% mc, the starch resonance (102 ppm for C1 and 71 ppm for C2–C5) showed a $T_{1\rho}$(H) value of 5.7 msec, which was lower than the corresponding values in the heated starch component at 2.0% mc (Table III). Even though the resonance at 71 ppm consisted of contributions from both components, it was predominately contributed from signals of the starch component. The resonance for the C6 carbons overlapped more significantly with that of wheat gluten peaks and, thus, was not used for the $T_{1\rho}$(H) measurement. The gluten peaks also showed a $T_{1\rho}$(H) of 9.3 msec (171 ppm) and 9.6 msec (29 ppm). A similar trend was found for the mixture at 20.0% mc. Values of $T_{1\rho}$(H) for the heated mixture were higher than those of the heated individual components (Table III). At 2.0% mc, $T_{1\rho}$(H) values for the heated mixture of 9.3 msec (171 ppm) and 9.6 msec (29 ppm) were more than twice as high as the corresponding $T_{1\rho}$(H) values of the heated gluten at 2.0% mc (i.e., 4.3 msec [171 ppm] and 4.9 msec [29 ppm]). These great discrepancies suggested that starch and gluten are mainly phase-separated, and that the molecules are experiencing different environments. Kalichevsky and Blanshard (1992) reported that starch and gluten are not miscible with one another, based on a glass transition study. For the heated mixture at 20.0% mc, the $T_{1\rho}$(H) values for gluten and starch remained more similar to those in individual components (Table III). $T_{1\rho}$(H) for gluten and starch mixtures decreased with increasing moisture content. Thus, water played a significant role in plasticizing the polymers in the heated mixture, reducing the $T_{1\rho}$(H).

The higher water dependence of gluten $T_{1\rho}$(H) in the heated mixture was probably partly due to a strong affinity of gluten to water. Similar conclusions were also reported from water self-diffusion behavior in starch-gluten mixture (Umbach et al 1992). The results from this study suggest that at 20.0% mc for the starch-gluten mixture, water was associated with gluten and, thus, was not available for the starch. The $T_{1\rho}$(H) of both components in the mixture behaved individually (Table III). In the absence of water (e.g., 2.0% mc), it is possible that gluten might be associated with starch molecules through hydrogen bonding at the interface. This polymer-polymer association, even though small in population, might be effective in hindering the molecular mobility and thus, was believed to be responsible for the relative slow relaxation [long $T_{1\rho}$(H)] for the dried mixture (2.0%).

TABLE III
Proton Rotating Frame Relaxation Times [$T_{1\rho}$(H)] of Starch, Wheat Gluten, and Starch-Gluten Mixtures Heated at 100°C/10 min

		$T_{1\rho}$(H) (msec)			
		Starch		Wheat Gluten	
Sample	mc (%)	102 ppm	71 ppm	171 ppm	28 ppm
Starch	2.0	3.3	3.2	...	...
Gluten	2.0	...	...	4.3	4.9
Gluten	20.0	...	...	3.3	3.6
1:1 mixture	2.0	5.7	5.7	9.3	9.6
1:1 mixture	20.0	4.2	4.2	3.6	3.7

Temperature Dependence of Wheat Gluten $T_{1\rho}$(H)

To further explore the effect of water on heated gluten, $T_{1\rho}$(H) was studied at various temperatures. Figures 7 and 8 show the $T_{1\rho}$(H) values for heated gluten with 2.0 and 20% mc as a function of temperature. Both samples showed a decrease in $T_{1\rho}$(H) as the temperature decreased from ambient to below the freezing point. For dried samples (2.0% mc), the $T_{1\rho}$(H) decreased as the temperature decreased and reached a minimum at –13°C (Fig. 7). There was also a small shoulder in the $T_{1\rho}$(H) at ≈12°C. For the samples with 20% mc, the minimum in $T_{1\rho}$(H) shifted to <–33°C (exact temperature for the minimum not obtained because of the limitation of the NMR instrument), and the small shoulder in $T_{1\rho}$(H) was shifted to ≈–3°C (Fig. 8).

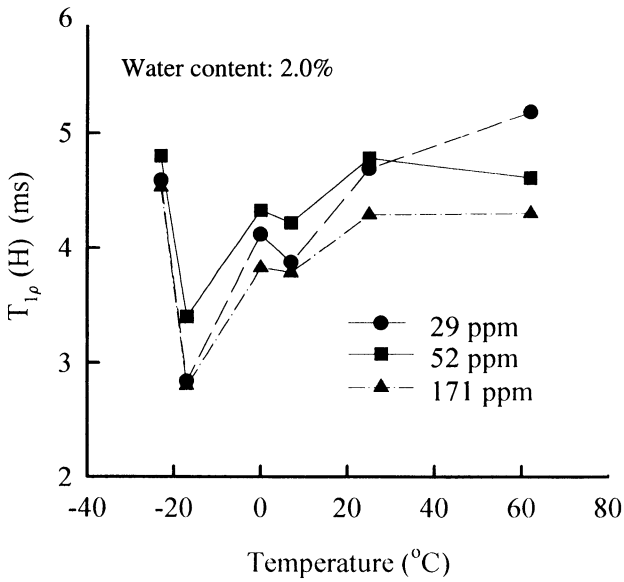

Fig. 7. Proton rotating frame relaxation times [$T_{1\rho}$ (H)] of heated wheat gluten at 2.0% water content as a function of temperature. Lines are for presentation clarity.

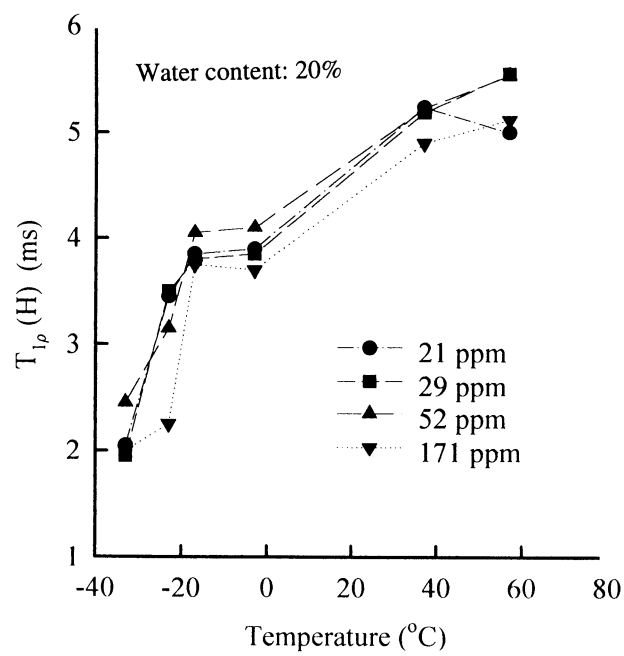

Fig. 8. Proton rotating frame relaxation times [$T_{1\rho}$ (H)] for heated wheat gluten at 20.0% water content (MC) as a function of temperature. Lines are for presentation clarity.

Note that these data are for gluten samples subjected to a stepwise temperature treatment, while data in Table II are from one-step cooling to 20°C. In view of the difference in sample thermal history (and molecular changes in gluten) in these two sets of experiments, comparison in $T_{1\rho}$(H) at 20°C between Figure 8 and Table II is not possible, although the values were within the same general range (i.e., 3–5 msec). Because the values at 20°C in the plots in Figure 8 are missing, it is not clear where the 20°C values are, but Table II data suggest that they could be in the 3.3–3.9 msec range. However, this might not be the case, as the sample has a different thermal history.

The $T_{1\rho}$(H) value corresponding to a simple molecular motion associated with correlation time τ can be described as:

$$1/T_{1\rho}(H) \propto \omega\tau/(1 + 4\omega^2\tau^2) \quad (4)$$

where ω is the spin-lock frequency. The temperature dependence of correlation time τ can be described by the Arrhenius equation:

$$1/\tau \propto \exp(-E/kT) \quad (5)$$

where E is the activation energy, T is the absolute temperature (K) and k is the Boltzmann constant. The change in molecular mobility with temperature thus can be observed from $T_{1\rho}$(H). Theoretically, $T_{1\rho}$(H) decreases with increasing τ at low temperature (solids) to a minimum when $2\omega\tau = 1$. $T_{1\rho}$(H) then increases with increasing τ values in a high temperature limit (liquid). Thus, the temperature at the minimum is the temperature at which the correlation time of the molecular mobility can be obtained from the spin-lock frequency.

In biopolymers the molecular mobility is complicated, and there are many kinds of motion occurring simultaneously. The observed $T_{1\rho}$(H) would represent the superposition or combined contributions of many of such processes (Dickinson et al 1988). A τ of 0.1 msec was estimated from the minimum in Figure 7. The weak shoulder in the $T_{1\rho}$(H) in Figure 7 indicated that complicated relaxations were involved in the $T_{1\rho}$(H) of gluten. The result in Figure 8 indicated that as the water increased to 20.0%, the temperature was <–33°C in order for the gluten protons to experience molecular mobility in the similar time scale (0.1 msec). In other words, at the same temperature, the protons in the 20.0% gluten would experience molecular mobility in a shorter time scale than that of the 2.0% mc gluten. It is apparent that molecular mobility in gluten is strongly dependent on water. Notice that the observed changes in $T_{1\rho}$(H) to a minimum are not likely to be identical with the thermomechanical glassy-rubbery transition, as these temperatures were at least 50°C lower than the T_g range reported earlier for gluten (Kalichevsky and Blanshard 1992, Cherian and Chinachoti 1996). The onset of mobility associated with $T_{1\rho}$(H) may be mainly from contribution of the sidechain protons of the protein.

SUMMARY

We have discussed the proton rotating frame relaxation times of native and heated samples of waxy corn starch and wheat gluten. Heating resulted in a decrease in $T_{1\rho}$(H) for both the starch and gluten. It was suggested that the decrease in $T_{1\rho}$(H) was due to the transition from a crystalline order to an amorphous structure. $T_{1\rho}$(H) of gluten was sensitive to moisture content. A similar effect was also observed for gluten in the 1:1 starch-gluten mixture but not in starch. The $T_{1\rho}$(H) of gluten showed a minimum at –13°C for the 2.0% mc sample and <–33°C for the 20.0% mc sample. Wide line ^{1}H NMR showed very little or no mobile water in gluten at <7.5% mc. The results might suggest the association of water with gluten. The distinctively different $T_{1\rho}$(H) of starch and gluten in the heated mixture indicated that the two components were macroscopically phase-separated.

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