Mobility of "Unfreezable" and "Freezable" Water in Waxy Corn Starch by ^{2}H and ^{1}H NMR

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"Freezable" and "unfreezable" water in waxy corn starch were characterized by thermal analysis, and the mobility in those states was characterized by solid state ^{2}H and ^{1}H NMR. Water was found to be isotropically mobile for samples over a range of water contents (6.3–47% by total weight) at room temperature. Mobility increased with increasing water content and temperature. According to ^{1}H and ^{2}H NMR data, a large fraction of "unfreezable" (DSC) was relatively mobile comparable to a liquid state even down to −32 °C. Some anisotropically immobile D_2O at low temperatures exhibited a solid state Pake pattern in ^{2}H NMR spectra, which was similar to that of frozen D_2O (ice) with a 144 kHz splitting. The decreasing fraction of mobile water with decreasing temperature suggested that only some of the so-called "unfreezable" water could be progressively immobilized as temperature decreased. However, much of the water (>50% of water present) remained very high in mobility, regardless of the relatively rigid starch molecules in the glassy solid state.

Keywords: *Water; freezable; freezing; mobility; NMR; nuclear magnetic resonance; unfreezable water; bound water; water activity; glass transition; starch*

INTRODUCTION

It has been recognized that many properties of food polymers, such as glass transition temperatures and enzyme activities, are strongly dependent on their interaction with water [e.g., Rockland and Stewart (1981) and Slade and Levine (1991)]. The dynamic processes involving water–polymer interactions in food, however, are not well understood. Water in food can be described by three properties or parameters: water content, water activity, and water dynamic mobility. Water activity has been used extensively as an empirical measure of the degree of water "binding" (Bone et al., 1975; Scott, 1957; Franks, 1982). However, it has been suggested that water activity is not the parameter that directly affects food stability (van den Berg and Bruin, 1981; Slade and Levine, 1991; Franks, 1982). Rather, it has been recognized by many that food properties are more related to the dynamic molecular mobility "state" of water in foods [e.g., van den Berg and Bruin (1981)].

The dynamic behavior of water has been studied using nuclear magnetic resonance (NMR) [e.g., Hills et al. (1989, 1990), Belton et al. (1991), Yakubu et al. (1990, 1991, 1993), Chinachoti (1993), Chinachoti and Stengle (1990), Kim-Shin et al. (1991), and Cherian and Chinachoti (1996)]. The slowing down of water motion at low water content has been reported to be associated with "bound" water arising from hydrogen bonding (Leung et al., 1983; d'Avignon et al., 1988). In terms of molecular sorption, monolayer and multilayer water have been attributed to bound water, but they are poorly defined without a direct measurement of the molecular dynamics. In a higher moisture system, water is

expected to move freely and undergo rapid motion, with a rotational correlation time τ_c at a picosecond scale (Bryant and Shirley, 1980; Bryant, 1987). Bound water in food polymers was often characterized by its NMR spin–spin relaxation time (Fuller and Brey, 1968; Steinberg and Leung, 1975; Nagashima and Suzuki, 1984; Richardson et al., 1986). A two-phase, fast exchange model of "free" water and bound water usually fits well for systems in the high-moisture range, e.g. >40% (Schmidt and Lai, 1991; Richardson et al., 1987a,b; Callaghan et al., 1983). In a lower moisture range, however, information in the solid state is limited.

The bound water and the glass transition concepts have been debated in recent years (Levine and Slade, 1988, 1989; Simatos et al., 1989; Blanshard and Franks, 1987). On the basis of the dynamic behavior of water in food polymers, bound water, referred to by some as "unfreezable" water, was suggested not to be energetically bound to polymer chains in any equilibrium sense but rather to result from kinetically retarded diffusion of water molecules in a metastable glassy solid state (Levine and Slade, 1988, 1989). For instance, in sugars, the residence time of water at a certain site was found to be extremely short (<1 ns) (Franks, 1983a,b). The notion that the retarded diffusional motion of water is due to the high local viscosity in the glassy state has gained much attention, even though experimental data directly relating water mobility and glassy–rubbery states are not yet available in food polymers. Other studies showed that water was mobile in nylon 6 (Jin et al., 1984), epoxy resin (Moy and Karasz, 1980), and polymeric carbohydrates (Jin et al., 1984; Wynne-Jones and Blanshard, 1986). The actual state of water in food polymers, whether it is chemically bound or kinetically retarded, is still being debated, and the question will not be resolved until more experimental data on water

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dynamics become available. Limited data are available on water in solids, but solid state NMR has been expanded in recent years to study water in polymers (Hey et al., 1990; Yakubu et al., 1990, 1991, 1993). Magic-angle spinning, wide-line, and high-resolution ^1H NMR spectra for water/starch systems revealed heterogeneity in proton mobility (Wu et al., 1992). Fitting a two-component model has been reported to show that the proton mobility was dominated by rigid components (from macromolecules) and mobile water protons (Wu et al., 1992). Solid state ^2H NMR using deuterated water is another promising technique for the study of water dynamics. In epoxy resins (Jin et al., 1884), water was found to be virtually free despite a strong tendency for the formation of hydrogen bonding. Yakubu et al. (1990) studied the mobility of D_2O in potato starch, using the same technique, and found that water underwent anisotropic motion at some water content (<62.5% water, total basis).

Despite the recent progress in studies of water mobility, its relationship with thermomechanical (glassy–rubbery) properties in food polymers is not yet well understood. This type of information is crucial in our fundamental understanding of the role of water dynamics and glass transition in controlling food stability. It is our objective to investigate water mobility by ^1H and ^2H NMR in solid and semisolid systems over a range of water contents from low ("unfreezable") to high ("freezable").

EXPERIMENTAL PROCEDURES

Sample Preparation. Waxy corn starch (Staley Waxy No. 1) was obtained from A. E. Staley Manufacturing Corp. (Decatur, IL). Three treatments were used: (1) variation in moisture or D_2O contents (for ^1H or ^2H NMR analysis, respectively), (2) variation in temperature for starch hydrated with D_2O to 9.3% D_2O content, and (3) starch in excess D_2O (60% D_2O content), gelatinized, and cooled stepwise to desired temperatures.

A given amount of H_2O or D_2O was mechanically mixed (stirring by hand with a spatula) with a known amount of dry sample (<2% moisture). The samples were stored for 2–7 days at ambient temperature to allow equilibration before the NMR analyses. Hydration was repeated by equilibrating with saturated salt solutions of known relative humidity, to achieve similar moisture contents (Greenspan, 1977). No significant experimental difference was found between the two hydration methods. The final H_2O and D_2O contents of the samples were measured by thermogravimetric analysis (TGA), which was earlier confirmed by a standard vacuum oven drying method (Li et al., 1996a).

Thermal Analysis. Crystallized (freezable) water and noncrystallized (unfreezable) water in starch were measured using a Perkin-Elmer DSC-4 differential scanning calorimeter (Perkin-Elmer, Norwalk, CT) equipped with an internal cooling system and nitrogen gas purge. Approximately 20 mg of sample was hermetically sealed in a hermetic DSC pan (Perkin-Elmer) and cooled from 15 to −40 °C at a cooling rate of 2 °C/min.

It is recognized that many investigators have chosen enthalpy of melting in the heating mode to calculate for the freezable water fraction, although the cooling mode may sometimes be used in comparison with other techniques [e.g. Korber et al. (1991)]. For this particular work, we chose to measure the heat of fusion upon cooling due to a number of reasons. One of the reasons was to keep the DSC experiment parallel with the NMR experiment (see below), which was also done by cooling. We were limited on the cooling capacity of the NMR spectrometer, and quench cooling samples by liquid nitrogen and then heating was prohibited. Therefore, the cooling mode was used both for the NMR and DSC experiments.
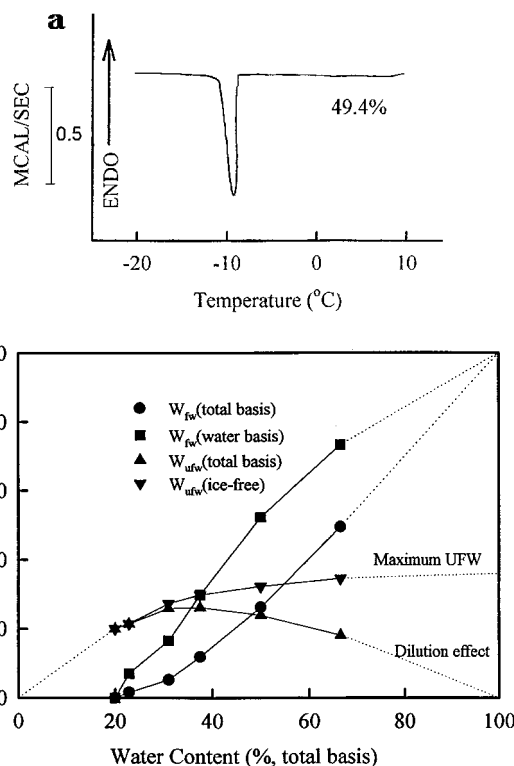

Figure 1. (a) DSC cooling thermogram for waxy corn starch with 49.4% water content. (b) Freezable and unfreezable water in waxy corn starch, as a function of water content.

The total concentration of unfrozen water in starch was approximated from the exotherm of ice formation

$$W_{ufw}(total) = \frac{g \text{ of unfrozen water}}{g \text{ of (water + starch)}} \quad (1)$$

with

$$\frac{g \text{ of unfrozen water}}{g \text{ of water}} = \frac{\Delta H - \Delta H_0}{\Delta H_0} \quad (2)$$

where $\Delta H_0 = 79.9$ cal/g is the heat of fusion for pure water at 0 °C and ΔH is the heat of fusion for the samples. It should be kept in mind that the approximated amount of unfreezable water was calculated using ΔH_0 for pure water at 0 °C and may be subject to some error since heat of fusion varies with temperature, e.g. $\Delta H_{-20°C} = 68.7$ cal/g. It has been pointed out by Hatley et al. (1991) that the calculated unfrozen water using the conventional means (ΔH_0 of pure ice at 0 °C) may be subject to error when the value is divided by a small value of solids (to calculate grams of unfrozen water on a solid weight basis). In our case, the amount of starch was over 20–70% range, and we did not attempt to calculate on a solid basis. Additionally, calculated values in Figure 1b are used to compare the entire range of concentration on a relative basis, and we felt that this conventional method serves the purpose here. Readers should be warned, however, of any future use of the values as absolute values. It should be noted also that experimental data from DSC are affected by kinetics and thus may be limited to the experimental conditions (e.g. cooling rate, sample size, moisture content, etc.) used here.

When ice is formed and separated in phase from the rest of the aqueous environment, it is important to explore the water content of the unfrozen phase (unfrozen water and starch). This may provide further information about the starch–water thermodynamics. We call this the local concentration of unfreezable water or W_{ufw} (ice-free), which was calculated as

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$$W_{ufw} \text{ (ice-free)} = \frac{\text{g of unfrozen water}}{\text{g of (unfrozen water + starch)}} \quad (3)$$

which is the percentage of unfrozen water expressed on an ice-free basis. Similarly, the total ice concentration can be expressed as

$$W_{ice}(\text{total}) = \frac{\text{g of ice}}{\text{g of (water + starch)}} \quad (4)$$

and the local concentration of ice can be expressed as

$$W_{ice} \text{ (water)} = \frac{\text{g of ice}}{\text{g of water}} \quad (5)$$

Percent error (reproducibility) for values was within ±5% variation.

NMR Analysis. Both the ^{1}H and ^{2}H NMR spectra of the samples were obtained using a Bruker ASX 300 NMR spectrometer (Bruker Instruments, Inc., Billerica, MA).

The ^{2}H NMR spectra were obtained using a $(90°-t_1-180°-t_2-\text{acquisition})$ pulse sequence with a 2.0 ms 90° pulse and a 15 ms t_1 interpulse delay. Two hundred milligrams of samples was used for each ^{2}H NMR experiment. The ^{1}H NMR spectra of water were obtained using a 2.5 ms 90° pulse and a 325 000 Hz spectral width. The large spectral width was necessary to observe both the wideline resonance of the solid protons and the narrow resonance in the samples (Wu et al., 1992). Approximately 300 mg of sample was used for each ^{1}H NMR experiment.

The sample temperatures during the NMR experiment were controlled by a Eurotherm device with −73 °C dewpoint air passed through a heat exchanger, both cooled by a Neslab chiller. Samples were cooled slowly in the NMR probe to various temperatures (27, 17, 7, −3, −13, and −23 °C), in a stepwise fashion, with a cooling rate of 2−5 °C/min. After an equilibration period of at least 15 min, the NMR spectra of the samples, at temperatures in the range between 27 and −40 °C, were obtained. The temperatures were controlled within ±0.5 °C.

RESULTS AND DISCUSSION

Physical States of Water. The physical states of water in food polymers are normally referred to as the solid, liquid, and vapor states. In food polymers, water in the adsorbed phase has been in the past described in a number of ways, e.g. bound, solid-like or ice-like, unfreezable, and trapped water [e.g., Rahman (1995)]. The term "freezing" of water refers to the crystallization of water (ice formation) on cooling to subzero temperatures. In this paper, we use both terms (freezing and crystallization) interchangeably. Figure 1a shows a DSC cooling thermogram of waxy corn starch with 49.4% water content, and Figure 1b shows the fractions of freezable and unfreezable water as a function of water content. Crystallization of water in the drier samples (<20% water) was not detectable upon cooling using DSC, and the water present in these samples has been classified as unfreezable or unfrozen water (Slade and Levine, 1991; Hatley et al., 1991). At a higher water content, the ice content may be expressed on both the total weight basis [W_{ice} (total)] and a total water basis [W_{ice} (water)]. As shown in Figure 1b, both values increased nonlinearly with increasing water content and may converge at 100% water content (dotted lines, Figure 1b).

The unfreezable water content can be expressed on both a total weight basis [W_{ufw} (total)] and an ice-free basis [W_{ufw} (ice-free)]. On total weight basis, the amount of unfreezable water increased from 0% to a peak at 30% as the water content increased from 0% to ~35%. It then decreased gradually, as the water content further increased to 100%, approaching 0% (due to dilution effect).

Since ice is phase separated, unfreezable water content, expressed on an ice-free basis, reflects the true mixture between water and starch. As shown in Figure 1b, W_{ufw} (ice-free) was the same as W_{ufw} (total) for water content <20%, since in this range of water content no ice was formed and all the water present was unfrozen water. However, as the water content further increased above 20%, some water started to crystallize but W_{ufw} (ice-free) also increased, leveling off at some high water content (>50% water). By extrapolation to the asymptotic level, W_{ufw} (ice-free) reached the maximum value at ~37% wet weight basis or 57% dry basis. This result also indicated that the amount of unfreezable water in starch at an intermediate moisture was not a constant value, changing significantly with water content until it reached a saturated value at the asymptotic level. This is interesting and most important to the functional properties and stability of biopolymers.

The continued increase in unfrozen water beyond 20% water content indicated that the water−starch association changes gradually and continues to increase as some excess water also gradually becomes phase separated and freezable. There is no clear-cut point at which unfrozen water completely stopped increasing, but at >50% water content the value changed very little. Thus, the notion that unfreezable water content of starch being at some given constant value, e.g. 20% (based Figure 1b), is not very accurate. The asymptotic value of ~37% fell in the range of values for maximum sorbed water of waxy corn starch, earlier reported to be 34% (Hellman et al., 1952). This unfreezable water has been described by a number of researchers as "plasticizing" water (Slade and Levine, 1991), and it may be associated more closely with the starch molecules (although its molecular mobility may remain high; see later details).

Because crystallization is a kinetically controlled process, the observed freezing was likely to be specific to the experimental conditions used. We repeated the DSC experiment at various cooling rates (2−10 °C/min) and found that the cooling rates affected the onset temperature of crystallization rather than the enthalpy, and the relative differences among samples have been confirmed. Various cooling rates in this case did not affect the conclusion.

Water Mobility by NMR. Solid State ^{2}H NMR. In solids, the deuterium NMR spectra are dominated by the quadrupolar spin interactions (Spiess 1985; Wu et al., 1992). For isotropic materials, the NMR frequency ($\delta\nu$) is given as (Abragam, 1961)

$$\delta\nu = (3/4)\pi\Delta(3\cos^2\theta - 1)/2$$

where $\Delta = e2qQ/h$ is the quadruple coupling constant and q is the angle between B_0 and the orientation of X−D bonds. Thus, each orientation of the X−D bond gives a resonance at a certain frequency. For systems in which the molecules undergo rapid rotational motion, the line shape of the spectrum is dependent on the molecular motion. In a fast rotational limit, only the time-averaged resonance can be detected, resulting in a single Lorentzian peak while, in a slow rotational limit, a Pake pattern of solid powder (<144 kHz split) will be observed (Mehring, 1983).
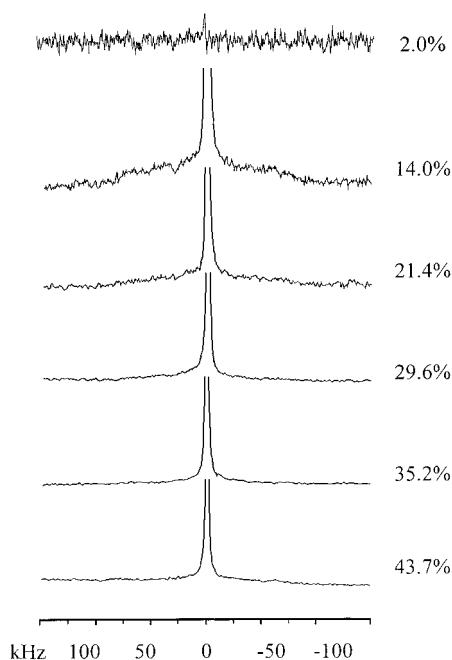
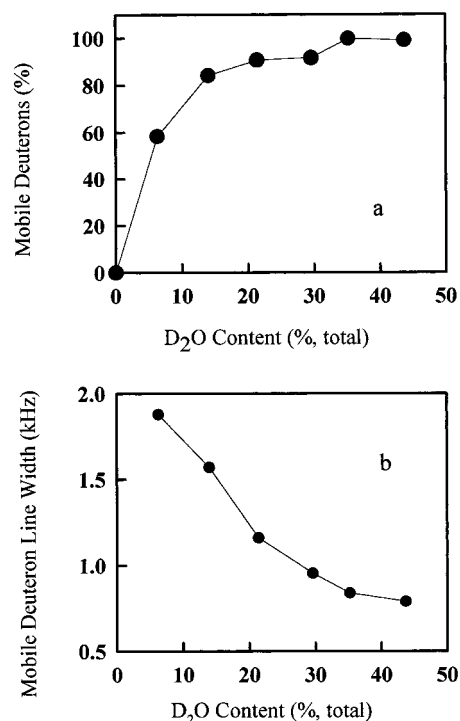**Figure 2.** ^{2}H NMR spectra for waxy corn starch of various D$_2$O contents at ambient temperature.

Figure 3. (a) Percentage of mobile water for waxy corn starch as a function of water content. The data were obtained in integration of the isotropic peaks in the ^{2}H NMR spectra in Figure 2. (b) Line width of the isotropic peaks in the ^{2}H NMR spectra for waxy corn starch as a function of water content.

Figure 2 shows the quadruple echo solid state ^{2}H NMR spectra for waxy corn starch of D$_2$O content ranging from 14.0 to 43.7% (total weight basis). The sharp peaks at 0 kHz correspond to the resonance of deuterons undergoing rapid isotropic motion. A weak powder (broad) pattern is seen with a <144 kHz width (more prominent at 14% D$_2$O content). This approximated width was found to be consistent with the theoretical calculation for resonance (144 kHz) of immobile deuterons (Weisser, 1980) and apparently did not change with water content. Baianu and co-workers reported ^{2}H NMR data for potato starch (Yakubu et al., 1990, 1991, 1993). They found that some water was anisotropic in motion but quite mobile (splitting only in the order of 2 kHz). For the case of corn starch reported here, the room temperature samples showed almost no sign of anisotropic motion. The sharp center peak (Figure 2) suggested that the main population of D$_2$O was highly rotationally mobile (closer to liquid water than to ice) even at D$_2$O content as low as 14%. When dried, no peak was observed at all, suggesting that the ^{2}H spectra were due to ^{2}H in D$_2$O and not natural abundance of ^{2}H in starch. The ^{2}H NMR spectra were also obtained 2−7 days after equilibration time, and no significant dependence on equilibration time was observed.

The quantitative measurement of mobile D$_2$O in the starch systems can be complicated by chemical exchange between the deuterons of D$_2$O and exchangeable hydroxyl protons of the starch molecules. To evaluate the effect, starch samples were mixed with D$_2$O at various concentrations and stored for up to 2 weeks to allow any possible exchange of deuterons with starch protons. Water was then thoroughly removed by vacuum drying (<2% moisture). The starch deuterons that might have appeared from the exchange process were then measured by ^{2}H NMR. The results showed no such deuterons on the starch molecules.

It can be seen from Figure 2 that the relative intensity of the Pake split component increased as the D$_2$O content decreased. The relative percentage of mobile

D$_2$O obtained by integration was plotted against D$_2$O content to show this increase in mobile fraction with increasing D$_2$O content (Figure 3a). At 6.3% D$_2$O content, ~58% of the D$_2$O was detected as mobile. This increase continued until a >90% level was reached at ~35% D$_2$O content, indicating that almost all of the D$_2$O molecules were mobile (rapid and isotropic in motion) at >35% D$_2$O content. Note that this value matched with the maximally sorbed water (34%) and the maximally unfrozen water (37%) discussed above.

Results from Figure 3a demonstrated a high mobility (liquidlike) of D$_2$O even at a relatively lower D$_2$O content (when starch might even be in the glassy state; see below). It was also seen from Figure 3a that there was 84% mobile D$_2$O present at 14% total D$_2$O content and 90% mobile D$_2$O present at 21.4% D$_2$O sample. This means that much of the sorbed moisture in the bound or multilayer water region is very rotationally mobile (isotropic, liquidlike) and only a small fraction of the D$_2$O exhibited low mobility at ambient temperature.

^{1}H Wideline High-Resolution NMR. Figure 4 shows the ^{1}H wideline NMR spectra for samples of various water contents. For a dry sample (containing <2% water) only a broad hump with 80 kHz width was observed. This broadness was due to dipole interaction of protons in the system. As the water content increased, the resulting spectra showed two distinct components, seen as the narrow and broad peaks of mobile and immobile protons, respectively. Other very small peaks were probably due to protons of other minor components or impurities. By deconvolution to separate narrow and broad peaks, the resulting broad spectrum was found to match in linewidth with dried starch (sample with <2% water content in Figure 4). This suggested that the spectra were a superposition of those from the rigid phase and mobile phase in the systems.

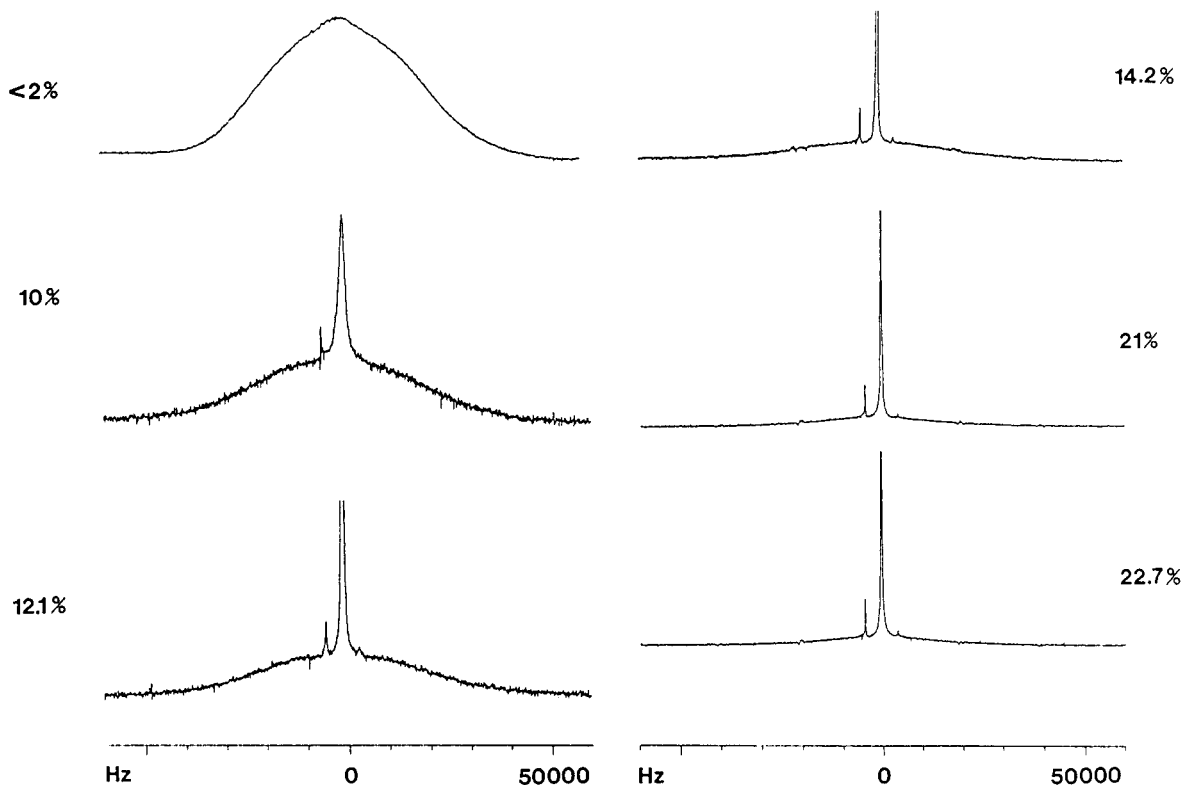**Figure 4.** ^{1}H NMR spectra for waxy corn starch at various water contents.
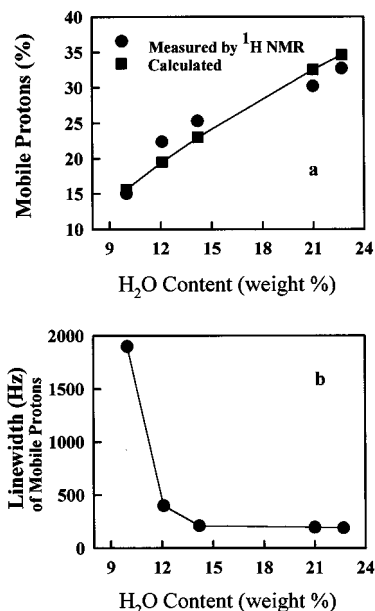

Figure 5. (a) Percentage of mobile water for waxy corn starch as a function of water content. The percentage was measured by the integration of narrow peaks spectra in (b). (b) Mobile proton linewidth of the ^{1}H NMR spectra for waxy corn starch as a function of water content.

The mobile proton signal intensity was measured by integration (Figure 5a). Similar to the case of ^{2}H NMR, the mobile ^{1}H signal intensity increased with moisture content. However, in this case both starch and water contributed to the ^{1}H signal. Thus, further analysis of the proton signals was needed. Assuming that all of the water present contributes to the mobile ^{1}H fraction, the expected mobile protons based on ^{1}H NMR data can be calculated from water protons/(water protons + starch protons). The calculated level of expected mobile protons is also shown in Figure 5a. The measured

mobile proton matched well with the calculated values in the samples. This result indicated that the assumption made might be correct; that is, the mobile proton signal was dominated by the proton from the water molecules. Even though water in this range of water content was considered unfreezable on the basis of DSC results (Figure 1), it exhibited a distinct higher mobility than starch molecules. This again supports our conclusion earlier that water was mobile in the starch matrix despite the extremely high rigidity of starch molecules in the solid and semisolid states. Such molecular rigidity has been observed in the same system by solid state ^{13}C NMR (Li et al., 1996a).

The line width is shown in Figure 5b. At water content >12%, the line width was only a few hundred hertz and relatively independent of water content. A dramatic increase in ^{1}H NMR line width was observed as the moisture content decreased below 12%.

Unfreezable Water Is Mobile in the Glassy State.
We have shown that waxy corn starch could hold up to $35 \pm 2\%$ amorphous (unfreezable) water, depending on its water content. The fraction of mobile water (liquid-like) in waxy starch within this water content range is high, and yet this water was unfreezable under the DSC experimental time and temperature. According to Levine and Slade (1988, 1989), unfreezable water could not be frozen into ice due to its being kinetically retarded in diffusional motion in the extremely viscous matrix of starch molecules. Thus, these water molecules should either have a very low mobility or be physically trapped in the starch molecules. The former possibility is not likely since our NMR data show a very high mobility (liquidlike). As discussed, only a small fraction of the ^{2}H NMR signal showed low molecular mobility, observed as the Pake pattern in the ^{2}H NMR spectra (Figure 2). A major fraction of D_2O is mobile in the rigid starch molecules partly due to the size
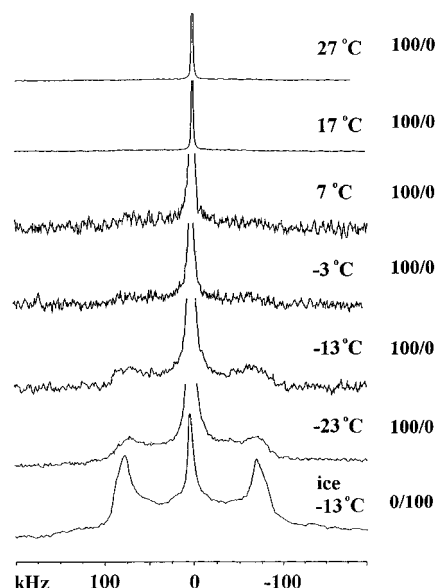
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Figure 6. ^{2}H NMR spectra for waxy corn starch with 9.3% D$_2$O at various temperatures.

difference between water and starch molecules. The high viscosity and rigidity of the starch molecules are caused by its connectivity of segments (Kuhn length), which might be many times the size of water molecules, leading to a great difference in molecular mobility. Unfreezable water is not by any means low in molecular mobility, earlier suggested to be caused by an extremely high local (molecular) viscosity (Slade and Levine, 1991).

Our data do not support the speculation that water molecules are physically trapped by the viscous matrix of rigid starch molecules with little free volume for diffusion. Even though moisture hysteresis is observed, it is also known from common practices that water can be readily removed by simple physical means such as vacuum- or freeze-drying. A similar point of view was also addressed by van den Berg and Bruin (1991). This indicates that water molecules in starch are not trapped in a vitrified state and can diffuse freely throughout the starch matrix (within the experimental time frame). Yet this mobile water is unfreezable under the DSC experimental time and temperature.

There are a number of possible explanations for the discrepancy between the NMR and DSC results. Since the two techniques measure very different properties at a very different time frame, it was highly possible that DSC was not sensitive enough to detect very small ice crystals and nuclei that developed upon lowering in temperature. Upon cooling to subzero temperatures (see detailed discussion below), some but definitely not all of the D$_2$O was immobilized and slowly exchanging with the rest of the D$_2$O (Figure 6). At -3 and -13 °C, the samples contained approximately 53% and 48% mobile deuterons, respectively (Figure 7). The remaining solidlike fraction gave a Pake pattern splitting similar to that of ice (Figure 6). Yet, much of the water (>50% of all D$_2$O present) remained in a highly mobile (liquid) state (over the temperature range studied), although the starch was in a glassy state.

A glass transition was not clearly observed by DSC in our study. However, gelatinized and native starches with <20% water content have been reported to have a glass transition above room temperature (Zeleznak and Hoseney, 1987; Levine and Slade, 1989; Kalichevsky et al., 1992; Vodovotz and Chinachoti, 1996) and, conse-

quently, the starch in the amorphous phase at 9.3% moisture can be assumed to be in a glassy state. Starch in this water range is rigid, containing a microcrystalline phase and a glassy amorphous phase (i.e., sample in Figures 6 and 7). The resulting strong resonance in solid state ^{13}C CP-MAS NMR spectra (Li et al., 1996a; Mora-Gutierrez and Baianu, 1991) was mainly due to the crystalline components, but at >40% water content, such spectra exhibited a poor signal-to-noise, indicating that the starch molecules were in a nonrigid, rubbery state.

The kinetics of ice crystallization has been explained in detail elsewhere [e.g., Franks et al. (1984) and Franks and Murase (1992)]. The conclusion that unfreezable water is mobile can be interpreted by its metastable state with starch (Li et al., 1996b). There are two competitive processes for water molecules to lower their energy during cooling. At a given temperature the net energy for change (chemical potential) is $\Delta G = \Delta H - T\Delta S$. In pure water, lowering the temperature below 0 °C causes the molecules to lose energy to keep $\Delta G = 0$, leading to fusion. Migration of water molecules to form nuclei or to deposit on crystal surface lowers the total energy by the enthalpy of fusion. In our systems, there was also an increase in randomness (of water molecules among the starch molecules), lowering the total energy by increasing ΔS or the entropy of mixing (hydrogen bonding exists in both cases but will not be considered here). That means the water can be in equilibrium ($\Delta G = 0$) without a large ΔH from freezing, i.e., $-T\Delta S$ balances out and water can be metastable. Crystallization of water upon cooling involves nucleation and growth, which are very sensitive to the water concentration. At a high water concentration, crystallization dominates and is spontaneous. As water gradually migrates away from starch molecules to form ice, the local water concentration decreases and the chemical potential of water due to the entropy of mixing changes from negative to zero at the maximum entropy of mixing. At this point the water molecules have no energy gain or loss, leaving the starch molecules before they enter the crystalline lattice. In other words, water at this concentration is metastable and crystallization is not spontaneous. It has been shown that unfreezable water in starch became metastable (at 20–30% moisture range) at subzero temperature and could not migrate into the water crystal lattice spontaneously (Li et al., 1996b). Thus, it is possible that water is unfreezable due to its metastable state and yet maintains liquidlike, high mobility.

There is one caution needed to be discussed for the quantitative ^{2}H NMR concerning the amount of mobile water. The relative intensities of the deuteron signals depend on the correlation time (τ) or motion of each deuteron in the structure and, therefore, the interpulse delay (Yakubu et al., 1990). Only if the deuterons are either in the fast motion limit, $1/\tau \gg \Delta$, or in the slow motion limit, $1/\tau \ll \Delta$, can the numbers of deuterons be represented by the intensity. In our experiments, it is more likely that the solid deuteron signals were affected by the interpulse delay. However, varying the interpulse delay over a 10–30 ms range showed similar results and the influence was not strong enough to affect our conclusions.

The mobility of the mobile water fraction of waxy corn starch changed with moisture content as expected (line width data Figures 5b and 3b). The ^{1}H NMR line width
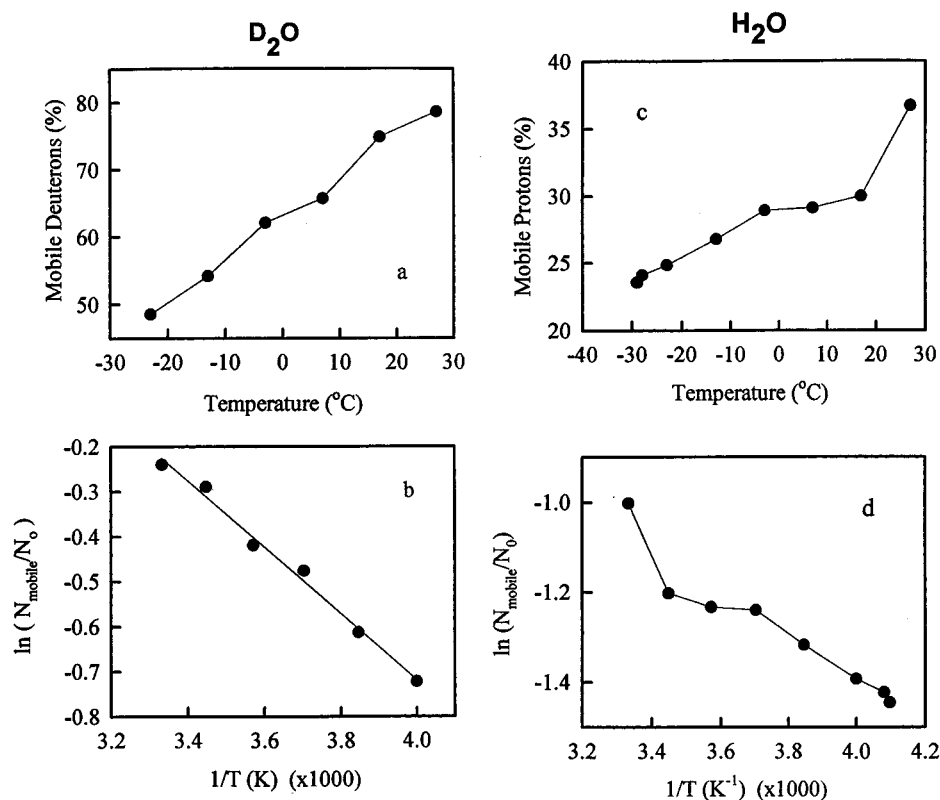
Figure 7. Mobile deuterons and protons for samples with 9.3% D_2O or H_2O content, respectively: (a and c) regular plots; (b and d) Arrhenius plots.

(Figure 5b) decreased more rapidly with increasing water content than the ^2H NMR line width (Figure 3b); both indicated a rapid increase in the mobility. Such information, however, can be complicated by chemical exchange and cross-relaxation phenomena commonly found in proton NMR (Koenig et al., 1978; Shirley and Bryant, 1982; Wise and Pfeffer, 1987). Therefore, a more specific and quantitative conclusion could not be made.

The apparent change of water mobility is complex as starch–water systems can be considered to have at least two phases or domains (Li et al., 1996b). The starch granules are semicrystalline and can form a multido-main structure upon absorbing water. As a result, water can be heterogeneous in mobility depending on its partitioning among domains, such as in a gel phase, in dissolved starch (solution domains), or in crystalline domains. Changing water content and temperature could greatly influence the balance or the distribution of the water in different phases or domains (Li et al., 1996b).

Water Mobility by NMR: Varying in Tempera-ture. Native starch at 9.3% D_2O and gelatinized starch at 70% D_2O were studied at various temperatures. Figure 6 shows the ^2H NMR spectra for the 9.3% D_2O content native starch sample cooled stepwise to various temperatures. The spectrum for pure D_2O sample frozen at -13 °C is used as a reference for solid Pake pattern of D_2O ice. The starch sample showed a resonance of mobile deuterons undergoing rapid isotro-pic motions (middle peak) with a small Pake pattern starting to develop at lower temperature due to im-mobility developed upon cooling. Note that the Pake splitting for the starch samples was virtually the same as that of D_2O ice (Figure 6). As the temperature decreased, the mobile deuteron peak intensity decreased

and its line width increased (Figure 7). On the other hand, the intensity of the Pake pattern increased as the temperature decreased, indicating that more D_2O was frozen (immobilized) in a solid state. As discussed earlier on the basis of the DSC results, all water in this moisture content is expected to be unfreezable. Yet, it was noted that this unfreezable water exhibited a much higher mobility than that of ice but some developed a solidlike character (Pake pattern at subzero tempera-tures). Our spectrum for pure D_2O ice showed a mobile isotropic signal possibly due to the presence of impuri-ties preventing a complete crystallization. ^1H NMR spectra for a similar sample hydrated with 9.3% water (Figure 7b) were found to support the same conclusion, i.e., the existence of both mobile and immobile compo-nents.

The results here are in agreement with previous work [reviewed by Steinberg and Leung (1975)] that a sig-nificant amount of water can remain isotropically mobile even at temperatures as low as -80 °C. It has been discussed (for the case of proteins) that such water could not freeze because it either interacts with protein surfaces (bound water), is trapped in small spaces within the matrix of solid samples, or is in a solution with dissolved protein molecules (Hansen, 1978). Using the same analogy, water in starch can be rather heterogeneous and can exist in different phases or domains. Our results have shown that water in waxy corn starch can be isotropic in motion with some molecular anisotropic mobility. However, the anisotro-pic population of water was found only in a low moisture content (<14% moisture and at subambient tempera-ture) at a much lower moisture level than the case of potato starch (Yakubu et al., 1991), for which anisotropy of water molecules was found at a higher moisture content (~60–62.5% total basis). Pake pattern width
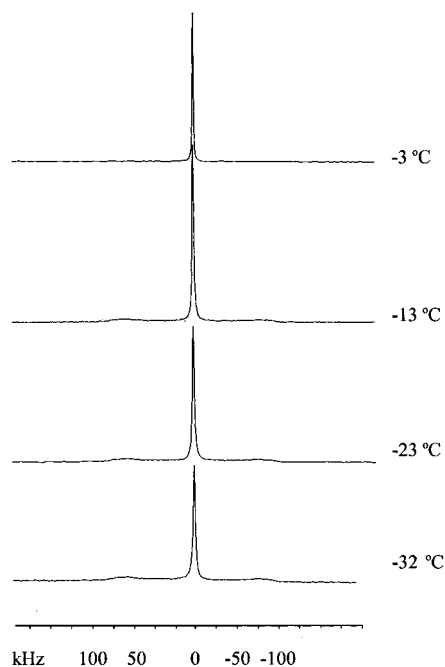
Freezable and Unfreezable Water by ^{2}H and ^{1}H NMR

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Figure 8. ^{2}H NMR spectra for gelatinized waxy corn starch of 60% D_2O content at various temperatures.

of <2 kHz in potato starch indicates that, even in the slow fraction, the water mobility was still very high, although they were slowly exchanging (on NMR time scale). In the case of waxy corn, we found a wider peak. In other words, different starches may result in different water behavior. In our case, most of the water was mobile and the free and the loosely bound water exchanged rapidly, resulting in the Lorentzian signal, while some anisotropic was observed (<14% moisture and subambient temperatures).

In poly(ethylene oxide), the anisotropic ^{2}H NMR signal (a few kilohertz split) was also found, and it was concluded on the basis of the two-component behavior in NMR data that the exchange process between freezable water and unfreezable water or bound component was quite slower on the NMR time scale (Hey et al., 1990). However, on the basis of our data much of the some of the unfreezable water was not rotationally bound and was moving and exchanging freely. This clearly shows that not all unfreezable water is bound.

Therefore, freezable and unfreezable waters have been referred to inaccurately by some to have a meaning on the molecular dynamic state of water (at least in starch).

An Arrhenius plot (Figure 7) of the percentage of mobile deuterons gave an E_a of 6.14 kJ/mol, similar to the heat of fusion of pure water. The decrease in mobile fraction and the increase in the solid Pake pattern fraction with decreasing temperature indicated the progressive freezing or immobilizing of some of the so-called unfreezable water upon cooling. In other words, even though the water was uncrystallizable under the DSC experimental time and conditions, only a fraction of it became increasingly immobile on the NMR time scale as the temperature decreased.

For the case of gelatinized waxy corn starch with 70% D_2O content, the ^{2}H NMR resonance for both mobile and solid deuterons was found to change similarly upon cooling to subzero temperatures (Figure 8). Similar Arrhenius plots of (Figure 9) this time showed a curvilinearity as the temperature decreased to -10 °C
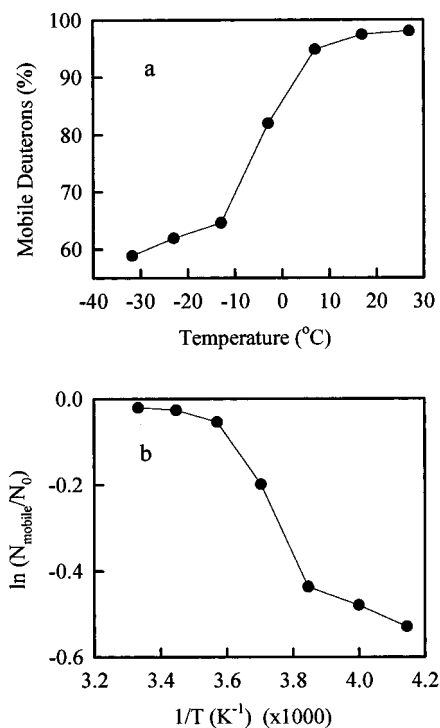

Figure 9. Mobile deuterons for gelatinized waxy corn starch with 60% D_2O content: (a) regular plot; (b) Arrhenius plot.

(due to a major ice formation). Further decrease in temperature led to further immobilization in a linear fashion to -32 °C.

Summary. The mobility of water for crystalline and noncrystalline phase in waxy corn starch was studied by ^{2}H and ^{1}H NMR. At subzero temperatures, unfreezable water, on the basis of DSC results, exhibited a high molecular NMR mobility even at -23 °C (with some fraction of anisotropic or immobile phase). It was found that the mobility and the fraction of mobile phase decreased with decreasing temperature and water content. As the temperature decreased below the freezing points, the DSC unfreezable water was found to be partly mobile with both isotropic (liquidlike) and anisotropic (solidlike) motions. The mobile fraction decreased with decreasing temperature. The activation energy calculated for immobilization of unfreezable water (NMR) was found to be the heat of fusion for pure water. ^{1}H NMR also confirmed the high mobility attributable to water in waxy corn starch, which is much more mobile than the starch protons. It is suggested that most of the water in waxy corn starch is highly mobile (isotropic, liquidlike) even though the material is in a solid semicrystalline, glassy state (9–24% water content). This means that water in the glassy state of starch can greatly influence reactions at both ambient and freezing temperatures. At least in this case the glassy state of the solid materials is not an appropriate term to imply or to predict the molecular dynamics of water and its influence on food stability.

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