^{2}H and ^{17}O Nuclear Magnetic Resonance Study of Water in Gluten in the Glassy and Rubbery State

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ABSTRACT

Water mobility in hydrated gluten at 0–50% mc was studied using ^{17}O and ^{2}H nuclear magnetic resonance (NMR). Glass transition behavior measured by dynamic mechanical analyzer (DMA) and by differential scanning calorimetry (DSC) was compared to the NMR signal intensity. The ^{2}H NMR signal intensity increased during glassy-rubbery transition due to hydration. ^{17}O NMR detected the signal when >0.21 g of water/g total occurred in the "free" or bulk water region, as determined from the sorption isotherm. Freezable water was only observed at >18% mc, when the sample was in the rubbery state (beyond the midpoint glass transition) and water was unfreezable in the glassy region. Results from the different techniques (NMR, DMA, DSC, and sorption isotherm) showed good correlation, although experimental conditions, sample preparations, and the time frame of each experiment were inherently different.

Hydration of wheat protein (gluten) greatly influences the mechanical and rheological properties of a dough (Finney and Shogren 1972, Mani et al 1992). The mobility of water is critical for the interaction of gluten and water to form a viscoelastic network. As a low molecular weight diluent, water acts as a plasticizer and increases the mobility of the system (Ferry 1980). It affects the functional properties by causing conformational changes allowing for hydrophobic interaction (Kinsella and Hale 1984) and also acts as a solvent for the hydrophilic and water-soluble components (low molecular weight gluten proteins) at low ionic strength. Hydration with mixing causes the unraveling and unfolding of the tightly packed aggregated gluten proteins and the formation of a viscoelastic network that contributes to optimum dough volume during baking (Hoseney and Rogers 1990).

Gluten proteins are the principal proteins occurring in wheat. Based on their solubility, they are broadly divided into two categories: 1) gliadins (soluble in aqueous alcohol) and 2) glutenins (soluble in dilute acid) (Bushuk 1985). Gliadins are relatively short chain proteins (molecular weight ranging from 25,000 to 100,000 Da) and are responsible for the extensibility and cohesive properties of gluten. Glutenins have a higher molecular weight than gliadins (ranging from 100,000 to 1 million Da) and contribute to the elasticity of gluten (Redman 1971).

The glassy-rubbery behavior of amorphous and partly crystalline foods has become an area of increasing interest (Noel et al 1990, Slade and Levine 1993). The glassy-rubbery behavior of gluten has already been reported (Hoseney et al 1986, Kalichevsky et al 1992a). The glass transition (T_g) of gluten fractions (glutenin and gliadin) has also been studied using differential scanning calorimetry (DSC) and mechanical spectrometry. Cocero and Kokini (1991) and deGraff et al (1993) reported that gliadins had a lower T_g than did gluten and glutenin, which was attributed to the lower molecular weight and the lower concentration of hydrophilic amino acids. Kalichevsky et al (1992a,b) and Kalichevsky and Blanshard (1992) reported the effect of lipids, emulsifiers, and sugars on the glass transition behavior of gluten as a function of moisture content. The effect of plasticizers on the mechanical and water vapor permeability barrier properties of wheat gluten films was studied by Gontard et al (1993) and Cherian et al (1995).

Water mobility has an important effect on the overall mobility and structural properties of metastable food polymer systems (Ablett and Lillford 1991). Nuclear magnetic resonance (NMR) techniques provide a powerful tool to study specific molecular interactions of water with other components. NMR has been widely used to examine water mobility by energizing chosen nuclei of water (^{1}H, ^{2}H, and ^{17}O) in various systems (Hills et al 1990, Belton et al 1991, Hills 1991). Details of the use of NMR to study water in foods can be found elsewhere (Richardson and Steinberg 1987, Belton 1990, Chinachoti and Stengle 1990).

^{1}H NMR has been commonly used to determine water mobility in various food systems. However, a major drawback of using ^{1}H NMR is the effect of the cross-relaxation process resulting in line broadening (Edzes and Samulski 1978, Shirley and Bryant 1982). The NMR data of a quadropolar nucleus (e.g., ^{2}H) is not affected by cross-relaxation, but may show the effect of chemical exchange, making the interpretation of the results difficult (Richardson and Steinberg 1987, Kakalis and Baianu 1988). The problems encountered with ^{1}H and ^{2}H NMR can be overcome using ^{17}O NMR, as shown in the studies with sucrose (Chinachoti and Stengle 1990), lysozyme (Kakalis and Baianu 1988), and wheat flour (Richardson et al 1985). Being quadrupolar, ^{17}O nuclei does not exhibit any cross-relaxation and its exchange rate is extremely slow. Proton exchange broadening in ^{17}O NMR can be easily eliminated by proton decoupling (Richardson and Steinberg 1987).

^{1}H and ^{2}H NMR were used to quantify the fractions of bound and free water occurring in wheat flour dough (Leung et al 1979, 1983; d'Avignon et al 1990). Attempts to quantitate the interaction of water with macromolecules using ^{1}H, ^{2}H, and ^{17}O NMR have been extensive (Kakalis and Baianu 1988, Otting et al 1991). Studies using these nuclei to relate the molecular level changes of macromolecules on hydration are fraught with problems because of the model system dependence and the proton-proton cross polarization effect on NMR line broadening (d'Avignon et al 1990, Belton 1991).

Richardson et al (1985) studied ^{17}O NMR water mobility and rheological characteristics of wheat flour suspensions and observed no direct relationship between water mobility and rheological properties. However, the wheat flour-water suspensions studied were at high moisture content (60–90% wb), which was not within the moisture range where glass transition occurs.

Belton et al (1988) applied Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence to study the proton transverse relaxation in dry gluten. There have been some studies on conformation of gluten and water structure using ^{13}C NMR (Baianu 1981, Belton et al 1985, Ablett et al 1988). Water mobility in gluten using proton NMR was recently reported by Kalichevsky et al (1992a).

Thermal techniques such as dynamic mechanical analysis (DMA) and DSC have been widely used to study glass transition

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behavior in various systems (Wunderlich 1990, Mathieson and Ibar 1991, Foreman et al 1992). For DMA, a stress is applied to the sample as a sinusoidal wave function with an increase in temperature. Depending on the nature of the material (viscoelastic character), the resulting strain frequency is either in phase with the stress (if the material is ideally elastic) or out of phase (if the material is viscoelastic). The extent to which the resulting strain is out of phase with the applied stress is given by the phase angle δ. The loss tangent (tan δ) is the ratio of E'' (loss modulus) to E' (storage modulus). Change in E', E'', and tan δ has been used to characterize the transition behavior. In this study, the peak of the tan δ dependence was used as the midpoint temperature (T_g). For the materials undergoing glass transition, changes observed in rheological properties are often greater than corresponding changes in thermal energy (Foreman et al 1992), and thus the DMA is usually more sensitive than the DSC.

Unfortunately, little work has been done to try to relate the molecular mobility (as influenced by water in this case) in gluten in relation to such changes in thermomechanical properties. Such a relationship would be critical to a more comprehensive understanding of gluten functionality. Therefore, the objective of this study was to investigate thermomechanical properties of wheat gluten in association with mobility as observed by NMR upon hydration.

MATERIALS AND METHODS

Materials

Wheat gluten from hard red spring wheat (Sigma Chemical Co., St. Louis, MO) with protein content of 80% db (N × 5.7) was washed with distilled water (1:3, w/v) and freeze-dried (Virtis Sublimator, model 50-SRC, Gardiner, NY). The sample was subsequently stored in a desiccant chamber (with phosphorous pentoxide) at room temperature. Deuterium oxide (99.9% pure) and 0.1% ^{17}O-enriched water obtained from Cambridge Isotope Ltd. (CIL, Cambridge, MA) were used for the NMR study. Salts used for the preparation of saturated solutions were all analytical grade (Fisher Scientific Co., Fairlawn, NJ).

Methods

A broad outline of the experimental approach used in this study is shown in Figure 1.

Differential Scanning Calorimetry

A Seiko DSC 100 (Seiko Instruments, Inc., Torrance, CA) was used for studying the thermal transition behavior of gluten. Low moisture samples (<20% mc), were prepared by equilibration with saturated salt solution at various relative humidities (0–97% RH) (Greenspan 1977). For the high moisture samples (>20%), water was directly sprayed to the sample (without mixing) and kept 25°C for 24 hr to equilibrate in a hermetically sealed container. After equilibration, samples were weighed (≈10–15 mg each) and sealed in stainless steel hermetically sealed pans (Perkin-Elmer, Norwalk, CT). DSC scans were performed from –80°C to 200°C at the rate of 5°C/min. The instrument was calibrated using mercury and indium. A glass transition is normally observed in DSC as a baseline shift due to a change in the heat capacity (Wunderlich 1990). The midpoint temperature of the baseline shift was used as the midpoint apparent glass transition temperature for this study. Samples were run in duplicate and the experimental error of measurements was within 3%.

For ice melting measurement, pure deionized distilled water was used as standard for the measurement of freezable water: % freezable water = (ice melting enthalpy of sample/ice melting enthalpy of pure water) × 100.

Dynamic Mechanical Analysis

The sample was prepared by mixing gluten and water (1:1.5, w/v) in a Brabender Farinograph (Brabender Instruments, South Hackensack, NJ) for 3 min. Sample (30 g) was pressed in a Carver

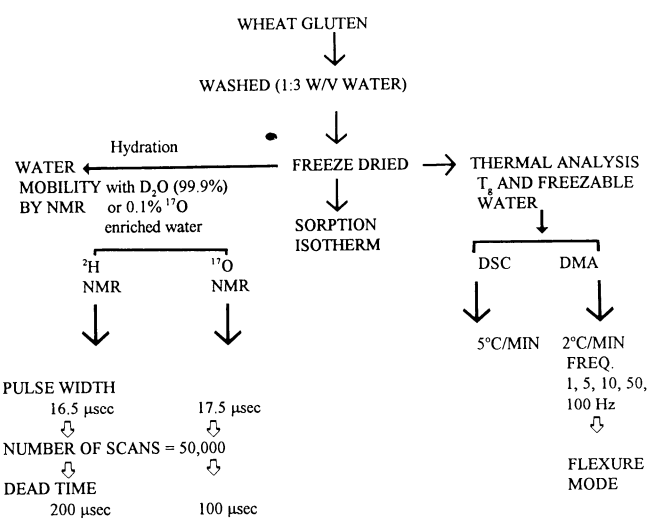

Fig. 1. Experimental approach used in the study of wheat gluten.

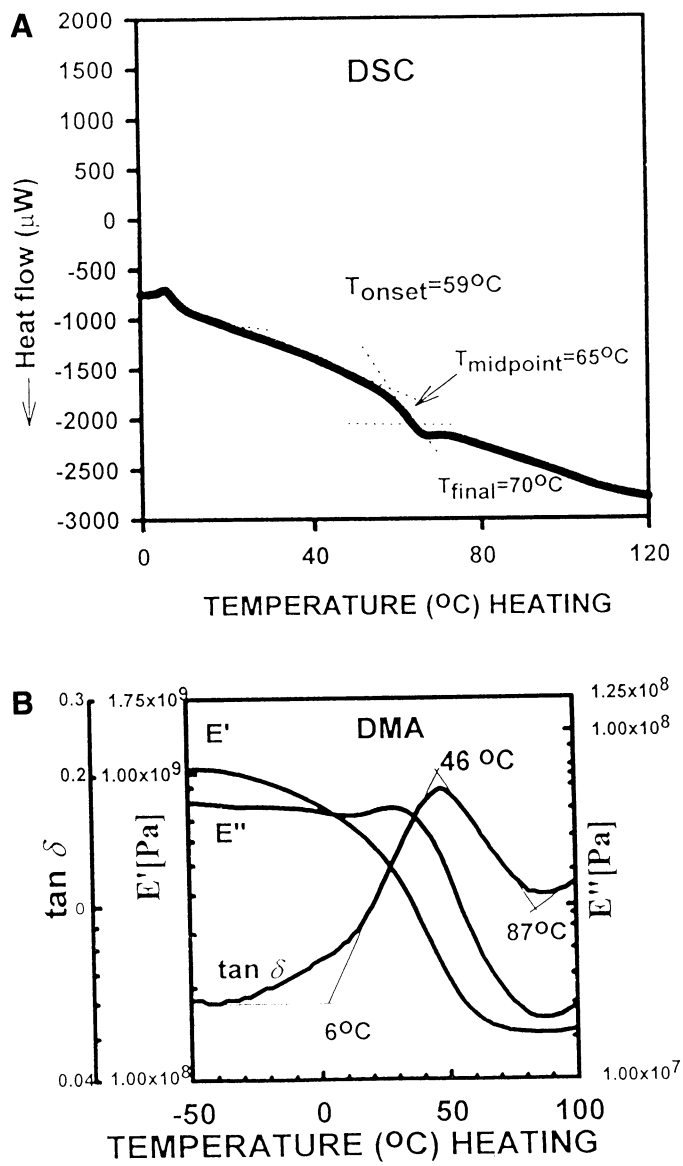

Fig. 2. Typical thermograms for vital wheat gluten: **A,** differential scanning calorimetry (DSC) at 13.5% mc; **B,** dynamic mechanical analyzer (DMA) at 15% mc.

press at 5,000 psi to form a sheet, then cut into rectangular strips (50 × 15 × 2.5 mm), and subsequently freeze-dried. Uniform geometry of the sample was critical to minimize variation in the results. Samples equilibrated to various moisture contents (as described for DSC) were then studied under the DMA (model 110, Seiko) using a three-point bending mode. Clamps were left untightened initially until the temperature reached –60°C. The sample was cooled further to –80°C and then scanned from –80 to 200°C at the rate of 2°C/min and at 1, 5, 10, 50, and 100 Hz frequencies. The strain level was within a 10-μm range. Experimental error of the tan δ peak T_g was within 6%, and measurements were made in duplicate.

Nuclear Magnetic Resonance

Water mobility of the samples was studied using ^2H and ^{17}O NMR. For ^2H NMR, deuterated water (99.9% D_2O) was used for sample hydration. For ^{17}O NMR measurements, 0.1% ^{17}O-enriched water was used. Samples were prepared as described above. NMR measurements were made on an NMR spectrometer (XL-300, Varian Instruments Inc., Palo Alto, CA) using 100 mg of sample. Pure D_2O and 0.1% ^{17}O-enriched water were used as references. For ^2H NMR, a 90° pulse width of 16.5 μsec and a recycle time of 0.05 sec. were used. For ^{17}O NMR, the pulse width was 17.5 μsec, recycling time was 0.02 sec, and ^1H decoupling was used. For both ^2H and ^{17}O NMR experiments, the dead time of the instrument was 100 μsec, and number of scans was <50,000, depending on the moisture content of the sample. Experiments were performed at a probe temperature of 25 ± 0.2°C. All measurements were done in duplicate and were within 5% experimental error.

References of increasing amounts gave increasing signal intensity; the standard curves gave a good linear correlation (R^2 = 0.98) for both nuclei used. These standard curves were then used to calculate the relative amount of water detected by NMR as percent of detected intensity (the signal observed in the sample divided by the signal expected to be observed, assuming that all of the water in the sample was bulk water). More detailed discussion of detected water can be found elsewhere (Chinachoti and Stengle 1990). Line width at half amplitude of the absorption spectrum was also determined.

Sorption Isotherm

Wheat gluten was equilibrated against saturated salt solution of known %RH (0–97%) (Greenspan 1977) in a minidessicator or so-called proximity equilibration cell (Lang et al 1981) at 25°C. After the first three days of equilibration, the samples were weighed daily until no weight change was observed for at least three consecutive readings. The equilibrated moisture content was determined by the weight gain using the vacuum oven method (AOAC 1984) at 60°C, 769 mm Hg, for 36 hr. All measurements were done in duplicate, and the experimental error on equilibrium moisture content was within 3% of the average moisture content.

RESULTS

Thermal Analysis Measurements

Typical thermograms obtained from DSC and DMA for wheat gluten are shown in Figure 2a and b, respectively. The change in the apparent glass transition midpoint temperature (T_g) with moisture content for vital wheat gluten is shown in Figure 3. The data in Figure 3 compares the midpoint temperatures of the two methods (DSC change in heat capacity and DMA tan δ peak at 1 Hz). The temperature range (onset to final) of these transitions are also shown as the shaded regions in Figure 4. In Figure 3, the midpoint T_g drops from ≈150°C at 0% mc to ≈20°C at 17–20% mc. This may be due to the plasticization effect of water (Cocero and Kokini 1991, Kalichevsky and Blanshard 1992). In general, the measured T_g agreed with values reported earlier for gluten (Hoseney et al 1986, Kalichevsky et al 1992a). However, at >0.15 g of water/g total, there is a slight deviation in values obtained from DSC and DMA (Fig. 3). The higher midpoint T_g of DMA in this moisture range was due to a shift in transition temperature due to a partial moisture loss in the DMA furnace as earlier reported by Kalichevsky et al (1992a).

There are some interesting points to be noted from this data. First, the midpoint T_g obtained by DMA (1 Hz) agreed quite well with that obtained by DSC, which was a surprising coincidence considering their differences in measured properties and the time frames of events being measured. But such agreement is not new and was noted earlier by other researchers (Ablett and Lillford, *unpublished data;* de Graff et al 1993).

As seen in the shaded regions of Figure 4, the transition range at a given moisture content was much broader for DMA (60–100°C wide) than it was for DSC (≈30°C wide). Because the heat capacity change was over a narrower temperature range than the changes in thermomechanical properties, DSC indicated a poorer

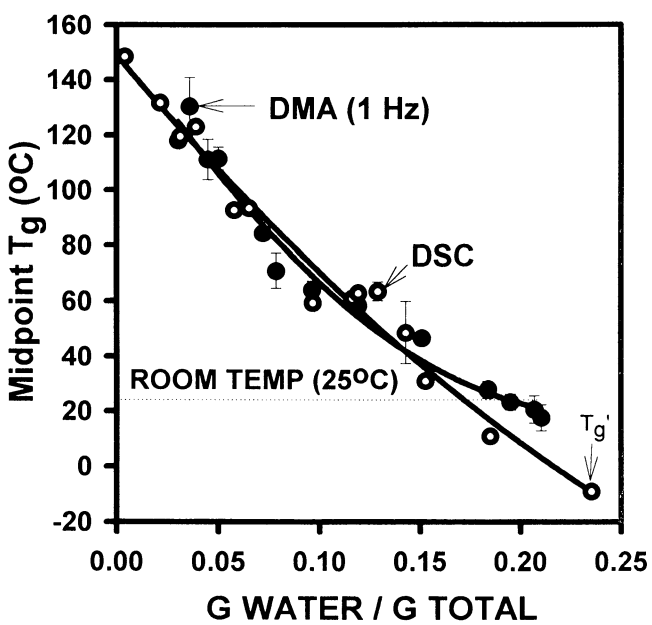

Fig. 3. Apparent glass transition midpoint temperature (T_g) changing with moisture content for vital wheat gluten as measured by differential scanning calorimetry (DSC) and dynamic mechanical analyzer (DMA).

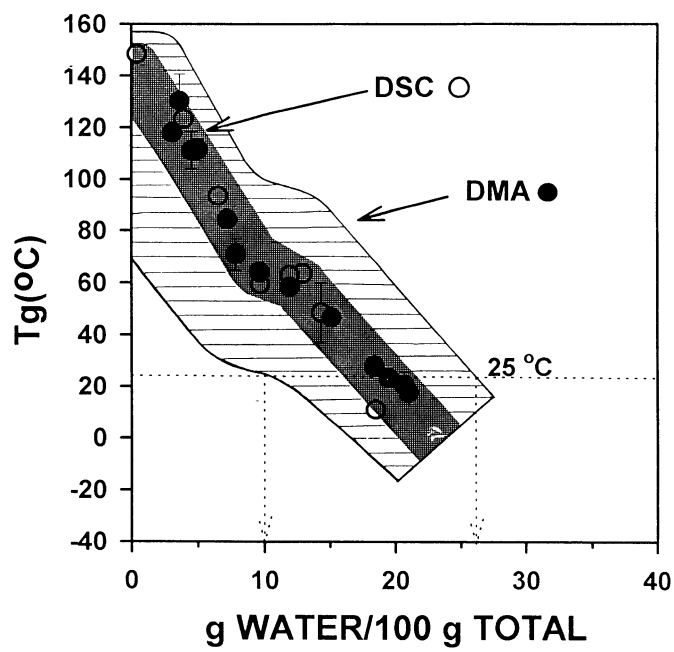

Fig. 4. Onset, midpoint and final glass transition temperature range (T_g) measured by differential scanning calorimetry (DSC) and dynamic mechanical analyzer (DMA).

sensitivity when compared to DMA. Therefore, DSC should not be used to precisely evaluate T_g for biopolymers. This problem is exacerbated at a lower moisture content. As was seen from the DMA data, samples with low moisture content exhibited a broader and weaker transition. Figure 4 indicates that even though the midpoint temperature agreed between the two techniques, the onset and final temperatures were far different. In this example, T_g values are confined to the particular method of analysis (and sample preparation). Comparison of measured values should be done with great caution, paying attention to detailed analytical procedure and data interpretation. In this case, discrepancies in the onset temperature values could vary by as much as 70°C (Fig. 4). There was a noticeable discontinuity in the onset, midpoint, and final transition temperature curves at ≈10% mc.

For practical food applications, it is important to observe changes in the gluten sample isothermally (25°C), as it is being hydrated. Such observed change can then be related to data most commonly used in food, such as water sorption isotherm and, in this particular case, the NMR water mobility, both at 25°C. Data from Figure 4 can be interpolated to obtain onset, midpoint, and final moisture content of the transition at 25°C (dotted line). These moisture contents were 10, 18, and 26%, respectively (Fig. 4). This represents a drop in E' from 7×10^8 Pa to 8×10^7 Pa as the sample was hydrated from 10 to 26% mc. Therefore, it was concluded that a room-temperature hydration of gluten resulted in a glassy-rubbery transition as the material passed over a 10–26% moisture range, with the midpoint of ≈18% mc (Fig. 4).

The DMA T_g range showed some frequency dependence, shifting to a higher temperature with increasing frequency. The energy of activation (E_a) of the transitions showed dependence on moisture; measured values were <450 kJ/mol, decreasing with moisture contents. However, the plot between E_a and moisture (not shown) was scattered with some indication of exponential relationship.

Since the range of moisture content studied here covers the range where the classical states of bound and free water or freezable-unfreezable water concepts apply, it would be interesting to examine the changes in water sorption parameters (monolayer from Guggenheim-Anderson-deBoer [GAB] model equation) (van den Berg 1985) and the NMR data.

Sorption Isotherm

The sorption isotherm for vital wheat gluten measured at 25°C is shown in Figure 5. A typical sigmoidal-shaped curve was observed and the data was fitted using the GAB model (van den Berg 1985):

$$M/M_0 = C_g \times k \times a/[(1 - k \times a)(1 - k \times a + C_g \times k \times a)]$$

where M_0 is monolayer moisture content, C_g is the GAB constant, k is a constant, a is water activity, and M is equilibrated moisture content.

The monolayer value was calculated to be 0.034 g of water/g of solids (C_g = 93.4 and k = 0.92; R^2 = 0.994). Upward concavity at >0.80 water activity (Fig. 5) occurred in the range of 0.12–0.15 g of water/g of solids (14–16% mc).

D_2O NMR

^2H NMR spectra obtained for all samples hydrated with D_2O showed a mostly symmetrical Lorentzian peak with signal-to-noise ratio of 4. The line width at half height was plotted against moisture (D_2O) content (Fig. 6) and showed an exponential decrease with moisture.

At a higher D_2O content (>0.25 g/g total), the observed line width was small (<200 Hz) and did not change significantly. However, at a lower D_2O content, the line width increased dramatically with decreasing D_2O content, particularly at <0.10 g of D_2O/g total. The line width increased from 1,000 Hz at 0.12 g of D_2O/g total to ≈6,000 Hz at 0.02 g of D_2O/g total. However, not all deuterium nuclei were detected by the NMR spectrometer. The detected signal, expressed in terms of the percent of detected intensity, was close to 100% only at higher D_2O content (>0.26 g of D_2O/g total). The percent of detected intensity dropped from ≈90% at 0.26 g of D_2O/g total to ≈0% at <0.10 g of D_2O/g total. However, even at <0.10 g of D_2O/g total, some D_2O signal was detected, although it showed a large degree of line broadening and deviation from Lorenzian line shape. The molecular interpretation of ^2H NMR data is complicated by the fact that ^2H on D_2O can rapidly exchange with exchangeable ^1H on the protein surfaces. Thus, if the exchange rate is rapid in the operating NMR time frame, the ^2H NMR signal represents a contribution of D_2O relaxation as well as relaxation due to the ^2H on the protein surfaces.

Comparative studies of ^2H and ^{17}O NMR relaxation of proteins (Picullel and Halle 1986) have shown that this is a major complication of ^2H NMR that does not exist in ^{17}O NMR. It is expected that as the moisture content is decreased, the protein experiences a considerable decreases in side chain mobility (Shirley and Bryant 1982, Kennedy and Bryant 1990). In addition, the amount of

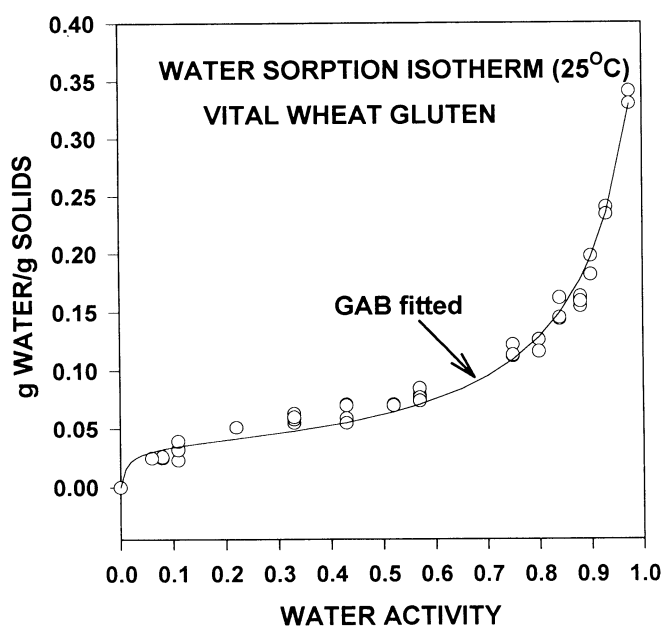

Fig.5. Sorption isotherm for vital wheat gluten measured at 25°C. GAB = Guggenheim-Anderson-deBoer model equation.

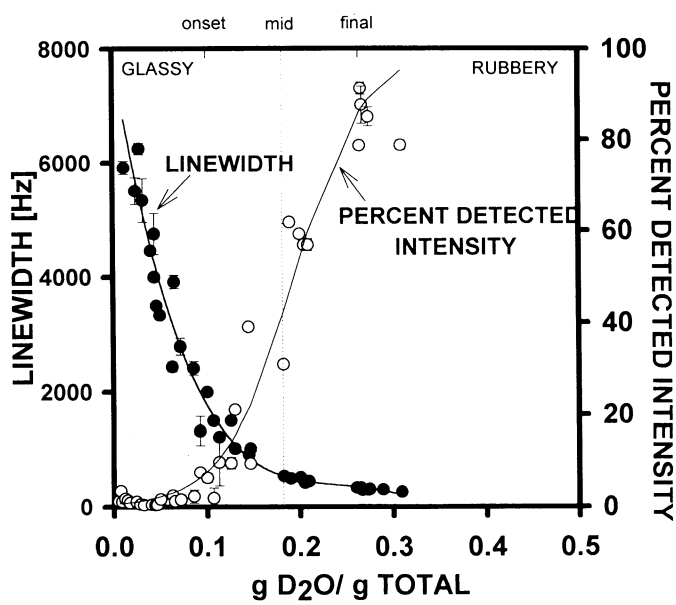

Fig. 6. ^2H NMR-detected line width and percent of detected intensity change with moisture content.

exchangeable protons would decrease. As the exchangeable protons cannot be accounted for in this experiment, the ^2H NMR intensity and line width data can only represent the exchangeable ^2H in the system. With limited information available in literature about the molecular dynamics of gluten, quantitation of the water dynamics from ^2H NMR alone is not possible.

^{17}O NMR

Previous work comparing ^2H and ^{17}O NMR response to water dynamics in various proteins clearly showed that ^{17}O NMR relaxation is far simpler in interpretation due to the absence of the contributions from nuclei exchange found in ^2H NMR (Halle et al 1981, Picullel and Halle 1986, Belton 1990). The wheat gluten samples were hydrated with ^{17}O-enriched water and subjected to ^{17}O NMR relaxation.

The ^{17}O NMR line width decreased with increasing moisture content (Fig. 7), indicating an increase in water mobility. The spectra showed a good signal-to-noise ratio at >31% mc. However, this only applied to the high moisture range (0.2–0.5 g of water/g total); at a lower moisture, the signal was too noisy and broad. The amount of water detected by NMR in this range was very small, as can be seen from the percent of detected intensity (Fig. 7). The extrapolation of the linear regression line to 0% detected intensity gave the x-axis value of 0.21 g of water/g total. This indicated that ^{17}O NMR could detect only the bulk or free fraction of water at >0.21 g of water/g total. Because the NMR spectrometer dead time could not be set at <100 μsec, the population with T_2 < 100 μsec could not be observed under experimental conditions (Chinachoti and Stengle 1990).

Freezable water was found only at >18% mc (Fig. 8), which coincides with the fact that ^{17}O NMR was detected only at >21% mc. This means that ^{17}O NMR detected the freely freezable or bulk water fraction. Because ^{17}O NMR is limited to free or bulk water, the ^2H NMR method was the only alternative in further analyzing the signals at lower moisture contents. From the ^2H NMR data, the change in percent of detected intensity with hydration could represent regions with varying system mobility (Fig. 4). At higher moisture content (>0.26 g of D_2O/g total) the system mobility was the highest. Thus ≈100% of the signals were detected and the line width was narrow. However, at <0.1 g of D_2O/g total, the slow system mobility resulted in a low detected signal.

Relationship Between ^2H NMR Mobility and T_g

DMA data (Fig. 9) for relative stiffness modulus at 1 Hz showed that the gluten started to go through a glass-rubbery transition upon hydration to 10% mc. The process continued through a midpoint transition at 18% mc and a final transition at 26% mc. This matches almost perfectly with the ^2H NMR detected intensity onset, midpoint, and final moisture (Fig. 9). The intensity change was fitted using an empirical model. Mathematical model fitting of the signal intensity data (following Peleg 1994) could be described as:

$$\% \text{ Detected intensity} = 100 \times \{1 - [1/(1 + \exp(M - M_c/a))]\}$$

where M is moisture content, M_c is critical moisture content, and a is the relative steepness of the concavity. The model fit, and the data correlated well (R^2 = 0.974 with data points used).

The calculated relative stiffness value obtained from E' data was also similarly fitted by an empirical equation as:

$$R(M) = [1/(1 + \exp(M - M_c/a))]$$

where $R(M)$ is the relative stiffness (E' at a given moisture/E' in a glassy state).

The model also fitted the relative stiffness data very well (R^2 = 0.98 with datapoints used). When compared, M_c and a from both cases (^2H NMR detected intensity and relative stiffness) were very close (from the ^2H NMR detected intensity: M_c = 19.4% and a = 3.8; from the relative stiffness data (at 1 Hz): M_c =19.5% and a = 4.1).

Therefore, the DMA and ^2H NMR data matched almost perfectly in the onset, midpoint, and final moisture content (25°C) for the glassy-rubbery transition. What is most interesting is that one method is based on a long-range analysis (structural, DMA) and the other is very short-range (molecular, NMR) This means that ^2H NMR can detect changes in D_2O and protein side chain mobility during plasticization (upon hydration). The surprising agreement between methods with such a different time frame seems to suggest that perhaps ^2H NMR data in the lower moisture (over the glass transition range) was rather more dominated by deuterons on the protein side chains (due to protons on the proteins exchanging with deuterons on D_2O), and thus the ^2H NMR signal was more

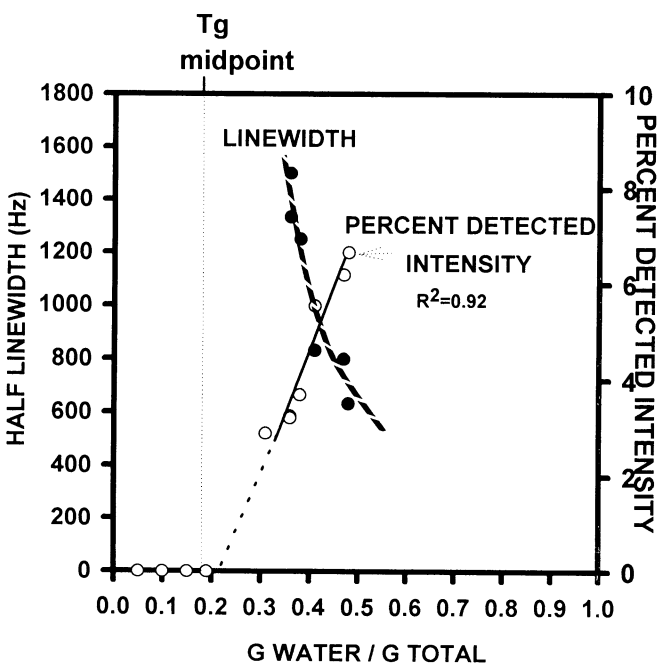

Fig. 7. ^{17}O NMR-detected line width and percent of detected intensity change with moisture content. Solid line is hand drawn for clarity.

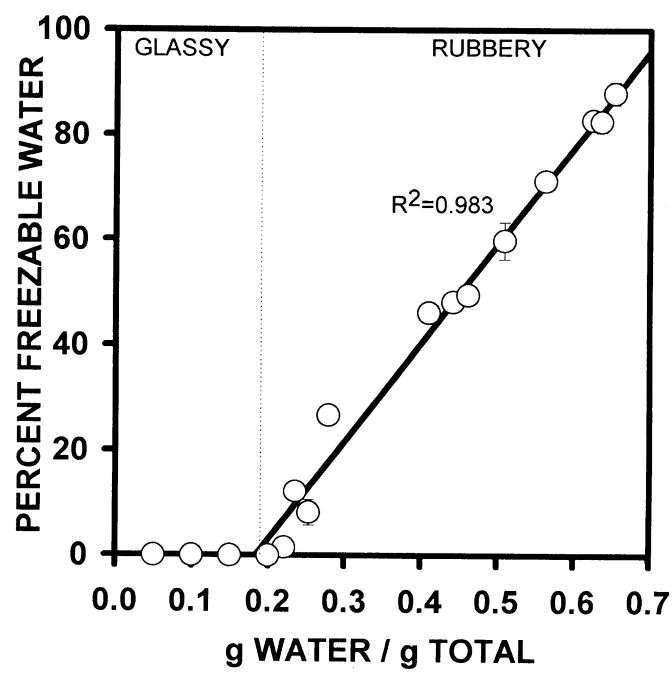

Fig. 8. Percent of freezable water at various moisture contents.

directly related to plasticization of the polymer side chains. This is the first time that NMR data for gluten has been in agreement with glassy-rubbery transition of gluten on the molecular level.

This means that the changes in glass-rubbery transition involve changes in the protein side chain and backbone mobility, which depend on the hydration and water mobility in neighboring regions. D_2O has been studied in terms of its influence on dough rheological properties as compared with H_2O (Hoseney 1979, Leung et al 1983). It was found that D_2O gave a stronger dough than H_2O. This has been attributed to a stronger hydrogen bonding in terms of the number of bonds and the bond energy difference between O-H---O and O-D---O units (Leung et al 1983). It is quite possible that these NMR observations may reflect the association between water and gluten, which is expected to have a profound impact on the mechanical property (E') upon plasticization that leads to a glassy-rubbery transition. Further experiments are being undertaken using solid-state NMR to characterize water in solids.

This data also confirmed that the glassy-rubbery transition upon hydration of gluten occurs over a broad moisture range (10–26% mc) rather than a sudden transition occurring over a narrower moisture range. It should be emphasized also that DSC, if used to identify the transition, would give a much narrower range in moisture over the glass transition (14, 17, and 19% for onset, midpoint, and final moisture, respectively). This is due to the poor sensitivity of the DSC technique and is not likely to have as much practical rheological meaning as values obtained from DMA.

DISCUSSION

The ^2H NMR relative intensity measurement agreed closely with the glassy-rubbery transition measurement by DMA. The effect of hydration was a lowering of the storage modulus during the increase in polymer mobility. Studies on other biopolymer systems, including cellulose, also suggested similar effects of hydration on polymer backbone motion (Yano and Hatakeyama 1988).

An increase in the detected intensity with the glassy-rubbery transition (Fig. 6) would suggest that the ^2H population relaxing beyond the dead time (100 μsec) increased when the material was converted to the rubbery phase. However, the interpretation of ^2H NMR intensity in complex systems is complicated by other factors, including: 1) the polymer proton exchanging with ^2H signal at different rates with changing moisture content (varying glassy-rubbery states), and 2) the D_2O existing in various states having varying relaxation times or varying mobility (Belton 1990, Hills 1991). Thus, the exchange rate and the D_2O mobility increase with increasing moisture content (Belton 1990, Hills 1991). The increase in moisture content also corresponds to a greater protein mobility (glassy-rubbery transition), which would account for the increase in relative population of ^2H signals detected.

^{17}O NMR intensity measurement showed an increase in the detected signal at >0.21 g of water/g total, indicating a highly mobile water fraction in this moisture range. Comparing the amount of freezable water (Fig. 8) with the ^{17}O NMR-detected free or bulk water, it is reasonable to conclude that ^{17}O NMR detects water that is phase-separated and undergoes rapid exchange in the rubbery region.

From the foregoing discussion, it can be suggested that part of the increase in the relative ^2H NMR signal (with glassy-rubbery transition change) could be associated with the increase in the D_2O as well as the protein side chain mobility in the rubbery region. The fact that the ^2H NMR signal intensity increased in parallel with the glassy-rubbery transition observed by DMA seems to suggest that ^2H NMR signals are influenced by the protein side chains going through a glassy-rubbery transition. Although it is difficult or impossible to separate the various contributions of the ^2H NMR relaxation, the overall relative ^2H NMR signal intensity was sensitive to the glassy-rubbery transition of the gluten as observed by thermal methods. Thus, the shorter time frame (NMR) relaxation observed was occurring simultaneously with the long-range structural relaxation (DMA and DSC).

A master curve combining the changes (^2H NMR percent of detected intensity, percent of freezable water, water activity, and relative stiffness) occurring in the gluten at room temperature with moisture is shown in Figure 9. This diagram clarifies the relationship of various phenomena occurring in gluten on plasticization by water even though the techniques probe different time scales. The information obtained from this data is crucial in deciding the moisture conditions for optimum functionality of the gluten.

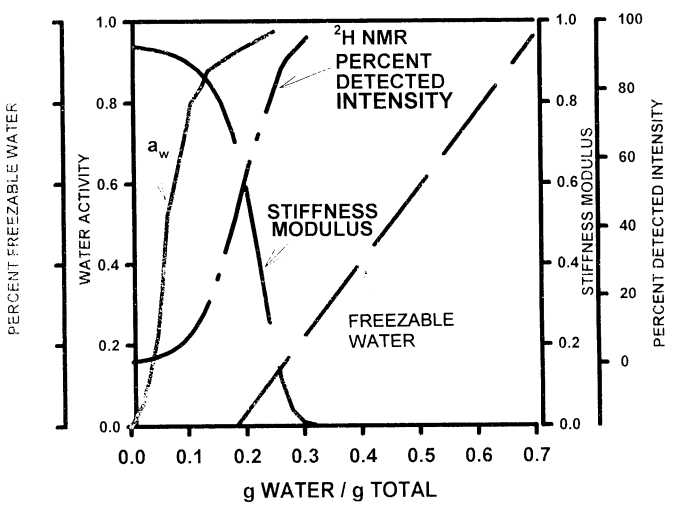

Fig. 9. Master curve for wheat gluten changes in freezable water, ^2H NMR-detected intensity, water activity (a_w), and relative stiffness.

CONCLUSION

The percent of detected intensity measured by ^2H NMR showed the changes in system mobility as the material was passing through the glassy-rubbery transition region. The signal intensity measured was strongly dependent on the physical state of the material. Mobility of water was detected at >0.15 g of water/g solids using ^{17}O NMR. The detected signal by ^{17}O NMR corresponded to that of the free water region on the sorption isotherm. Freezable water occurred also in this high moisture range. Unfreezable water in the lower range of moisture greatly influenced the system mobility in the glassy-rubbery state. The study also demonstrated an agreement in the results obtained from DSC, DMA, NMR, and sorption isotherm, in terms of molecular and structural changes occurring around a glassy-rubbery transition of gluten, even with a great degree of variation in experimental techniques and time scales.

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