Magic-Angle ^{13}C NMR Study of Wheat Flours and Doughs

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Doughs formed from hard- and soft-wheat flours have different rheological properties, which dictate their uses in baking. Cross-polarization, magic-angle spinning (CPMAS) ^{13}C NMR spectra of dry, hard- and soft-wheat flours show resolved signals for the starch and protein components of these composites, allowing differences in relative protein contents to be accurately measured. Proton rotating-frame relaxation measurements on these flours show that starch and protein components are phase-separated. CPMAS and Fourier transform (FT) MAS experiments on doughs formed by hydrating flours reveal that added water has little effect on protein signals of half of the hard-wheat flour, while added water causes nearly all of the main-chain protein signals in soft-wheat flour to disappear. Hard- and soft-wheat flour doughs also differ in the relative amounts of small, solubilized sugars and organic acids, which are observed in FTMAS experiments, and in the percentage of total starch that remains solidlike and is detected in CPMAS experiments. These differences are interpreted in terms of plasticization of the macromolecular components of flour by water.

INTRODUCTION

The hydration of wheat flour is an important step in the baking process. The mechanical and rheological properties of the resulting doughs affect the subsequent molding and shaping processes which are required to take flour into finished baked products (Matz, 1984). Hard-wheat flours, with their relatively higher protein content, are used for making breads and rolls, while soft-wheat flours are used in cookies and cakes. Water determines many of the mechanical and chemical properties of hydrated flours (Laidman and Wyn Jones, 1979; Bloksma and Bushuk, 1988; Levine and Slade, 1989), and it is likely that the differences in mechanical properties of doughs, as well as much of the chemistry of baking, are related to the interactions of water and flour.

More generally, water of hydration plays an important role in a wide variety of biological and agricultural systems. For example, lyophilized proteins contain only about 10% (by weight) "tightly-bound" water or structural water (Cole et al., 1988; Marchatti et al., 1985) and have reduced activity in the freeze-dried state. Only when these proteins are in their fully hydrated state, with a water content of nearly 40% (by weight), are they biologically fully active.

Nuclear magnetic resonance (NMR) spectroscopy has played an important role in understanding the interactions of water in a wide variety of biological and agricultural systems (Shirley and Bryant, 1982; Wise and Pfeffer, 1987), including wheat flours (D'Avignon, 1990). Most of this work has involved high-resolution ^{1}H NMR studies of water molecules. For the most part, these studies are able to detect and quantitate water in more or less mobile environments and are unable to address questions about immobilized or structural water's functionality.

Solid-state ^{13}C NMR has been used extensively to characterize the structure and dynamics of a variety of synthetic and naturally occurring macromolecular systems (Schaefer et al., 1984; Komoroski, 1986; Fedotov and Schneider, 1989). Studies of the structures of several different intact plant polymers, such as cellulose (Schaefer and Stejskal, 1976; Atalla et al., 1980; Earl and VanderHart, 1980) and lignin (Schaefer et al., 1981; Maciel et al., 1985; Lewis et al., 1987; Botto, 1988), have been reported. Solid-state ^{13}C NMR studies of 1,3-β-D-glucans (Saito et al., 1987, 1989) have provided insights into the secondary structures of these naturally occurring polysaccharides. NMR relaxation measurements can provide dynamical information about polymers and biopolymers in the solid state. Methodology developed for studying the structure and dynamics of synthetic polymers has recently been extended to study the dynamics of intact plant cuticle and its constituent parts (Zlotnik-Mazori and Stark, 1988; Stark et al., 1989; Garbow et al., 1989a, 1990).

Recently, we have described ^{13}C NMR work on dry hard-wheat flour and dough (Garbow and Schaefer, 1990). In the present paper, we extend our earlier magic-angle spinning (MAS) ^{13}C NMR study to include both hard- and soft-wheat flours and doughs. These solid-state ^{13}C NMR experiments permit the starch and protein components in the dry flours and their hydrated doughs to be observed directly and their relative proportions accurately measured. To investigate domain formation in these composites and provide a measure of the interaction between the macromolecular components of flour and added water, proton rotating-frame relaxation times, $T_{1\rho}(H)$, are measured. These relaxation times are sensitive to kilohertz-regime motions of polymer chains and have been used extensively to characterize motions in polymers (Stejskal et al., 1979). Here we show that $T_{1\rho}(H)$ can be used to investigate the distribution and dynamics of protein and starch components within flours and doughs.

MATERIALS AND METHODS

Sample Preparation. Dry, hard- and soft-wheat flours were kindly supplied by Dr. Hamed Faridi (Nabisco Brands) and were used without modification. Compositionally, the hard-wheat flour was 12.0 wt % protein, 13.5 wt % moisture, and 0.47 wt % ash. For the soft-wheat flour, the corresponding numbers were protein, 8.7 wt %, moisture, 13.0 wt %, and ash, 0.47 wt %. Doughs were prepared from these flours by hydrating them to approximately 40% water (by weight), stirring with a spatula to produce homogeneous mixtures, and then evaporating water under a stream of N_2 gas to the desired composition.

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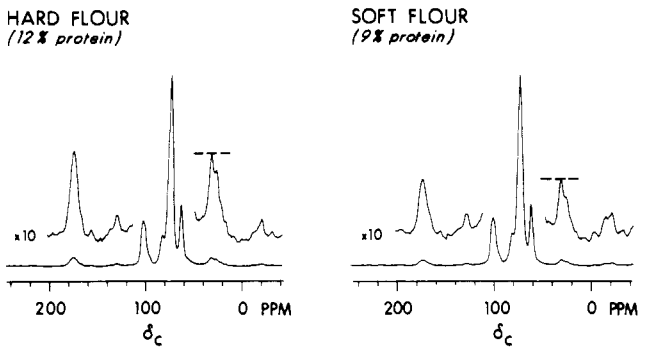

Figure 1. CPMAS ^{13}C NMR spectra of dry wheat flours: (left) hard-wheat flour; (right) soft-wheat flour. Spectra are plotted with equal heights for the 72 ppm starch peak. Line assignments in the CPMAS spectra are protein side-chain aliphatic carbon (δ_C 20–35), starch (δ_C 60–105), protein side-chain aromatic carbon (δ_C 130), and protein main-chain peptide carbonyl carbon (δ_C 175). The reduced protein content of the soft-wheat flour is indicated by the lower intensity of the aliphatic carbon signals (dotted lines).

Experimental NMR. ^{13}C NMR spectra were collected on a home-built, solid-state spectrometer operating at a proton resonance frequency of 127.00 MHz. Samples were spun at the magic angle (54.7°) with respect to the static magnetic field in a double-bearing rotor system at rates of 2 (wet samples) and 3 kHz (dry samples). A more detailed description of the magic-angle spinner can be found elsewhere (Schaefer et al., 1987). Sample weights in this study ranged from approximately 400 to 600 mg. Each ^{13}C NMR spectrum represents approximately 1 day of data collection.

Cross-polarization, magic-angle spinning (CPMAS) NMR spectra were collected with matched, 50-kHz, ^1H–^{13}C spin-lock contacts of 2 ms. High-power proton dipolar decoupling (50 kHz) was used in all CPMAS experiments. FTMAS ^{13}C NMR spectra were collected with low-power (5 kHz), continuous-wave ^1H decoupling. Recycle delays of 2 s ensured that these spectra were fully relaxed.

Proton rotating-frame relaxation times, $T_{1\rho}(H)$, are determined from the decay of carbon signal as a function of ^{13}C–^1H contact time (τ) in a CPMAS experiment (Stejskal et al., 1979). The ^1H relaxation experiment is performed in this way to take advantage of the spectral resolution of the ^{13}C NMR experiment. The value $\langle T_{1\rho}(H)\rangle$ is calculated from a straight-line fit of log(carbon signal intensity) vs τ, where τ varies from 2 to 8 ms.

RESULTS

Dry Flour. The CPMAS ^{13}C NMR spectrum of dry, hard-wheat flour is shown in Figure 1 (left). The major signals in this spectrum are due to sugar carbons in the starch component of the flours. Resonances due to both main-chain and side-chain protein carbons are observed in this spectrum, with signals due to C_α's of the proteins obscured by the tail of the 62 ppm starch resonance. Figure 1 (right) shows the spectrum of dry, soft-wheat flour. The 72 ppm starch resonance has been plotted at full vertical scale in each of these spectra, allowing the relative protein content of the two flours to be measured by the heights of the aliphatic carbon peaks (dotted lines). Because carbon signals due to the starch and protein components of the flours are well-resolved, relaxation experiments can be performed to probe the dynamics of specific structures. Table I lists values of the average rotating-frame proton relaxation time, $\langle T_{1\rho}(H)\rangle$, as measured through several of the resolved starch and protein carbon resonances in both the hard- and soft-wheat flour samples.

Hydrated Flour. Figure 2 shows both CPMAS and FTMAS ^{13}C NMR spectra of hydrated hard-wheat and soft-wheat flours (25 wt % H_2O). In these spectra, CP-MAS detects those components of the flour that are solidlike, while liquidlike components are observed in the FTMAS experiment. The spectrum of the hydrated hard-wheat flour is similar to that of the dry flour, with signals due to starch and protein components clearly visible. Relative to the dry-flour spectra, protein main-chain signals (δ_C 175) are about one-third of the total CPMAS ^{13}C signal for the hydrated hard flour and are nearly absent from the spectrum of the hydrated soft-wheat flour.

Table I. Average Proton Rotating-Frame Relaxation in Wheat Flour

	$\langle T_{1\rho}(H)\rangle$, ms			
	hard wheat		soft wheat	
signal	dry	hydrated	dry	hydrated
62 ppm (starch)	7.5	13.1	8.0	13.1
73 ppm (starch)	7.0	11.5	7.5	11.2
101 ppm (starch)	7.3	12.5	7.6	12.3
175 ppm (protein)	4.7	4.5	4.3	

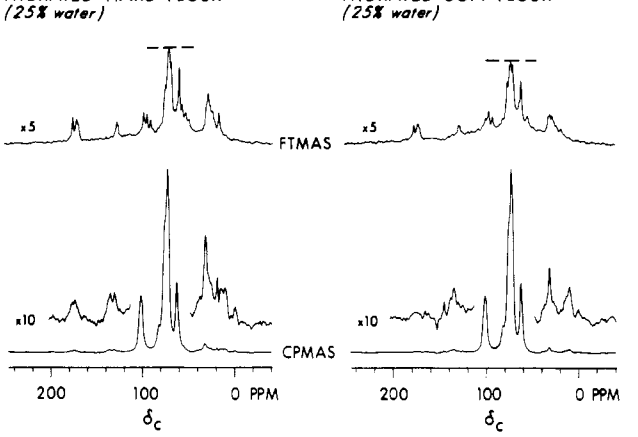

Figure 2. ^{13}C NMR spectra of hydrated wheat flours (25% H_2O by weight): (left) hard-wheat flour, (bottom) CPMAS, (top) FTMAS; (right) soft-wheat flour, (bottom) CPMAS, (top) FTMAS. Top and bottom spectra are plotted for direct intensity comparisons. Line assignments in the FTMAS spectra are aliphatic carbon (δ_C 15–35), oxygenated carbon (δ_C 60–110), olefinic carbon (δ_C 125–135), carboxylic carbon (δ_C 170–185).

Average proton rotating-frame relaxation times, $\langle T_{1\rho}(H)\rangle$, are summarized in Table I. These relaxation times are used both to evaluate the effect of water on the dynamics of hydrated flours (cf. below) and to permit comparison of CPMAS ^{13}C intensities for the starch component of the dry and hydrated flour samples. Taking into account differences in $\langle T_{1\rho}(H)\rangle$ values for dry and hydrated flours, approximately 45% of the total starch signal observed for dry hard-wheat flour is detected in the spectrum of the hydrated material, while 55% of the total starch signal is observed for the soft-wheat flour. Experiments have also been performed on flour samples hydrated with 35% water (by weight). The additional water produces no significant differences in spectra (data not shown) but does increase $\langle T_{1\rho}(H)\rangle$ for the starch by an additional 30%.

Signals in the FTMAS ^{13}C NMR spectra of the hydrated flours (Figure 2, top, left and right) arise from small molecules which are solubilized by the addition of water. Intensities in these spectra can be compared directly to one another (dotted lines) and to CPMAS intensities also shown in Figure 2. Hydrated, hard-wheat flour contains approximately 50% more solubilized small molecules per milligram of sample than hydrated soft-wheat flour. The relative percentages of small solubles do not increase for hydrated flours containing 35% water (by weight).

DISCUSSION

Dry Flour. Resonances due to starch and protein are clearly resolved in the CPMAS ^{13}C NMR spectra of the dry flours, allowing the relative amount of each material to be accurately measured. The relative intensities of the protein and starch resonances within each spectrum agree with the known compositional differences in protein content between varieties of hard-wheat (12% protein by weight) and soft-wheat (9% protein by weight) flour.

For macromolecules in the solid state, spin diffusion causes all of the protons to share a common $T_{1\rho}(H)$ relaxation time, as observed for each of the starch ^{13}C resonances in dry flour (Table I). Minor differences arise from the heterogeneity of starch in flour. The $\langle T_{1\rho}(H)\rangle$ for protein, measured through the carboxyl carbon, is 30% less than that of starch for both hard- and soft-wheat samples. This difference indicates that starch and protein have individual local dynamics and are separated into distinct domains. Spatial phase separation prevents spin diffusion from averaging $\langle T_{1\rho}(H)\rangle$ values for starch and protein to a common value. On the basis of the 10-ms time scale established by the $T_{1\rho}(H)$ NMR results, the protein domains must be at least 50 Å in size (Havens and VanderHart, 1985). Much larger domains are possible. The $T_{1\rho}(H)$ experiments do not distinguish between hard and soft dry wheat flours.

Hydrated Flour. Hydrating the wheat flours causes a number of small molecules to dissolve, and it is these that are observed in FTMAS ^{13}C NMR spectra of the doughs. The FTMAS spectra of Figure 2 are consistent with the dissolution of small sugars and organic acids. There are more such species (per milligram of flour) in the hydrated hard flour than in hydrated soft flour, possibly the result of higher starch damage of hard-wheat flour which would permit easier access of water in the 25–35 wt % range.

More solidlike than liquidlike species are present in both hydrated flours. For the hydrated hard-wheat flour, solidlike CPMAS ^{13}C NMR signals are observed from both the starch and protein components. Approximately 45% of the total starch present in the flour is detected. The remaining hydrated starch is not seen, presumably due to motional averaging which renders the high-power ^{1}H decoupling ineffective (Rothwell and Waugh, 1981). Similar motional effects have been observed previously in experiments on wet bacterial cells (Garbow et al., 1989b).

Results of the $T_{1\rho}(H)$ experiments indicate the starch and detectable protein components of the hydrated hard-wheat flour remain phase-separated. In addition, the dynamics of the detected solidlike protein components are largely unaffected by the addition of water, as indicated by the nearly equal values of $\langle T_{1\rho}(H)\rangle$ for the protein component in dry and wet samples. However, more than half of the protein is affected by the added water and is undetected by either CPMAS or FTMAS.

The interaction of protein with the added water in soft-wheat flour differs from that in the hard-wheat variety. Little protein signal is observed in the CPMAS ^{13}C NMR spectra of hydrated soft-wheat flours, and a consistently greater amount of the starch gives rise to a CPMAS ^{13}C signal in soft-wheat dough than in hard-wheat dough. We attribute the disappearance of protein signal to the effects of motional averaging, as mentioned above.

Plasticization of Flour. The rheology (processability) of milled flour is determined in part by starch-grain particle size and deformability (Bloksma and Bushuk, 1988). After milling, hard flours tend to have larger starch–protein particles that are rigid. Soft flours tend to have smaller starch–protein particles (or aggregates) that are more flexible (Bloksma and Bushuk, 1988). We believe that the NMR results support this sort of classification. The rigidity of the hard-flour starch–protein particles (relative to that for soft-flour particles) is associated with the reduced effect of water on protein components. Many of the proteins that are coating and attached to the starch–protein particle in hard flour are still solidlike. On the other hand, hydration of the soft-flour protein generates a new liquidlike component. This component may act as a compatibilizer in a heterogeneous composite, matching the properties of the plasticizer (water) to those of the filler (starch). The presence of a protein compatibilizer means that a more compliable, easily processed dough can be made from soft flour than from hard flour.

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