acid content and 17.95 °C from linoleic acid content.

As to the variation of fatty acid composition of seed lipid, it was known that some plants produce more highly unsaturated lipid when grown at lower temperature. The effect, however, appeared to be limited to certain species (Canvin, 1965). Our results show that buckwheat is temperature-sensitive plant for variation in fatty acid composition and lipid content of the grain. On the basis of this work, the early-seeding culture may be less desirable than the late-seeding culture for the storage because of higher lipid content and also higher temperature after harvest. However, the late-seeding buckwheat may be easily oxidized during storage period, especially for the ground flour, because of higher linoleic and linolenic acids. Increasing those essential fatty acids by the late-seeding time may be of nutritional importance.

Registry No. Arachidic acid, 506-30-9; behenic acid, 112-85-6; oleic acid, 112-80-1; linoleic acid, 60-33-3; linolenic acid, 463-40-1; eicosenoic acid, 28933-89-3; myristic acid, 544-63-8; palmitic acid, 57-10-3; stearic acid, 57-11-4; palmitoleic acid, 373-49-9.

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Mobility of Water in Wheat Flour Suspensions as Studied by Proton and Oxygen-17 Nuclear Magnetic Resonance

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The mobility of water in wheat flour suspensions and doughs (30–95% moisture) was investigated by nuclear magnetic resonance (NMR), in water and deuterium oxide. Two frequencies (20, 360 MHz) and two pulse sequences were employed for the proton (^{1}H) data; the standard 34 MHz and single pulse were used for the oxygen-17 (^{17}O) NMR data. The standard isotropic two-state model with fast exchange was used to interpret these data by means of the Derbyshire and Kumosinski models. The correlation time for the water "bound" by wheat flour was calculated to be 16.7 ps. The results suggested that the best NMR methodology for the investigation of water mobility in wheat flour suspensions was provided by ^{17}O NMR in deuterium oxide. However, both ^{17}O and ^{1}H NMR results showed the same trend in the dependence of the transverse relaxation rate on flour concentration in both water and deuterium oxide.

INTRODUCTION

In recent years one of the most successful techniques employed to investigate water binding and water mobility in biological systems is nuclear magnetic resonance (NMR) spectroscopy (Fuller and Brey, 1968; Steinberg and Leung, 1975; Hansen, 1976; Woessner, 1977; Ulmius et al., 1977; Eisenstadt and Fabry, 1978; Leung et al., 1979; Leung et al., 1983; Lang and Steinberg, 1983; Baianu et al., 1985). NMR spectroscopy provides a rapid, sensitive, noninvasive, and nondestructive determination of the molecular mobility of water in complex systems such as foods. Two NMR relaxation parameters, T_1 (spin–lattice or longitudinal relaxation) and T_2 (spin–spin or transverse relaxation) (Deslauriers and Smith, 1980), monitor a wide range of molecular mobilities of water in a macromolecular system. The complexity of such a system produces two major concerns: (1) the nucleus to be probed and (2) the model to be adopted for data interpretation.

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First, the majority of NMR studies of food systems have been carried out by probing the proton (^{1}H) nucleus. Recently, however, difficult problems with the interpretation of ^{1}H NMR relaxation data in macromolecular systems were pointed out; the major concern is with the relative influence of the various relaxation mechanisms that contribute to the line width of the water peak in the ^{1}H NMR spectra of such complex systems (Kalk and Berendsen, 1976; Edzes and Samulski, 1978; Koenig et al., 1978). These references stated that two relaxation mechanisms that contribute significantly to the water proton line broadening are (1) the cross-relaxation between the bulk of the water protons and protons of the macromolecule and (2) the proton exchange between distinct states of water, i.e. "bound" and "free" water.

Therefore, recent interest in measuring water binding and hydration of macromolecules has been directed to oxygen-17 (^{17}O) NMR (Halle and Wennerstrom, 1981; Halle et al., 1981; Laszlo, 1983; Lioutas, 1984; Lioutas et al., 1985b). The advantages of measuring ^{17}O are discussed by Halle et al. (1981); of major consequence is that "except for a narrow pH range around neutral, the ^{17}O relaxation is not influenced by proton (deuterium) exchange with prototropic residues on the protein". Therefore, the ^{17}O

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relaxation can only be affected by exchange of entire water molecules. Thus, the use of ^{17}O NMR eliminates the potential problem of cross-relaxation found in ^{1}H NMR. Since ^{17}O NMR measurements monitor directly the molecular motions of water molecules, the oxygen nucleus is the best probe for studying water binding, molecular motions of water, and the water–protein interactions in solutions (Halle et al., 1981; Lindman, 1983) and suspensions (Baianu et al., 1985; Lioutas, 1984; Lioutas et al., 1985b).

A variety of NMR techniques were previously applied to study hydrated wheat flour and its components (Steinberg and Leung, 1975; Capelin and Blanshard, 1977; Leung et al., 1979; Baianu and Forster, 1980; Baianu et al., 1982; Leung et al., 1983). In the recent study of Leung et al. (1983), water mobility in wheat flour doughs and breads was investigated by deuterium (^{2}H) relaxation NMR, which is not affected by cross-relaxation. However, chemical exchange of deuterium can occur, and this may cause a problem with the evaluation of ^{2}H NMR data.

Second, interpretation of NMR data is strongly model dependent (Finney et al., 1982). Different models often yield significantly different results. Currently, this model dependence is a major problem in the study of the hydration of macromolecules in biological as well as food systems. Further research is required to resolve this problem.

The primary objective here was to investigate the molecular mobility characteristics of water in the wheat flour system with ^{17}O NMR. High-field ^{17}O NMR and low- and high-field ^{1}H NMR relaxation measurements of wheat flour suspended in water or deuterium oxide, in the range of moisture contents from 30 to 95%, are reported here for the first time. The range of relatively low wheat flour to water ratios (60 to 95%) was emphasized in this study in order to probe the aggregation of flour particles in water; such aggregation is evidenced by both rheological behavior and NMR measurements at the same moisture content (Richardson et al., 1985b). From a technological viewpoint, however, ^{1}H NMR is more sensitive and less costly, and equipment for ^{1}H NMR is currently available for on-line applications in industry. A second objective of this work was, therefore, to compare the ^{17}O with ^{1}H NMR data and establish to what extent the latter could be used for water-binding studies in technological applications (Baianu and Richardson, 1983).

MATERIALS AND METHODS

Sample Preparation. The wheat flour was commercial grade, white, all-purpose flour. The flour was stored at 5 ± 2 °C. Its moisture content was 11.0% as determined by a modified air oven method; 130 °C for 6 h (AOAC, 1980). Protein content (N × 5.7) was determined by the micro Kjeldahl method (AOAC, 1980) to be 10.6%. pH measurements were made with a Beckman Research pH meter (Model 1019) with a standard electrode system (41263/39402) (Beckman Corp., Fullerton, CA). pH ranged between 5.30 and 5.50 for the wheat flour–water suspensions. pD ranged between 5.75 and 5.95 for the wheat flour–deuterium oxide suspension, calculated from the equation pD = pH + 0.45 (Covington et al., 1968).

The wheat flour–water suspensions and doughs at selected moisture contents were prepared by hand mixing at room temperature the appropriate amount of distilled water or 99.8% deuterium oxide (D_2O) with flour for 2.5 min. The replacement of D_2O for H_2O was done on a molar basis. The mixing time of 2.5 min corresponds to the optimum mixing time according to farinograph plots (AACC 1969) of flour at 47.5% w/w moisture content.

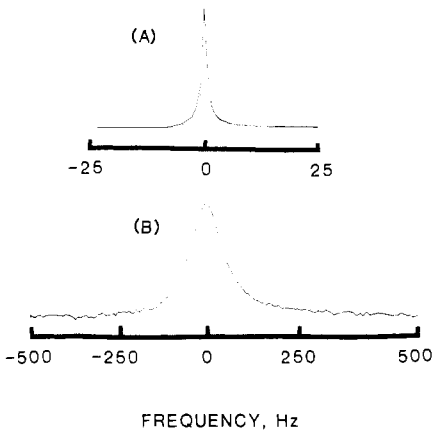

Figure 1. Spectra of a 90% moisture wheat flour–water sample: (A) 360-MHz proton NMR spin echo spectrum; (B) 34-MHz oxygen-17 Fourier transform NMR spectrum.

NMR Measurements. A NT-360 NMR spectrometer (Nicolet Technologies, Inc.), operating at 360.061-MHz proton resonance frequency, was used for the ^{1}H NMR measurements on samples made with either water (H_2O) or deuterium oxide (D_2O). Two multipulse techniques were employed for proton T_2 determination (Baianu et al., 1985). Data obtained with the Carr–Purcell–Meiboom–Gill (CPMG) (Meiboom and Gill, 1958) sequence were stored in a 32K point array, using an NIC 1180 computer. The Ostroff–Waugh (OW) (Ostroff and Waugh, 1966) pulse sequence consisted of a 90° pulse followed by a train of 90° phase-shifted pulses ($90\text{-}\tau\text{-}90_{90}\text{-}2\tau\text{-}...$ or X–Y–Y...). Pulses following the second pulse were equally spaced by a period of 2τ, with the observation of multiple-spin echoes between the pulses. OW spectra were stored in an 8K point array with an ADC of 12 bits. Arrays selected for each experiment provided adequate digitization and resolution. Pulse sequences were under computer control, using a 293 A′ pulse programmer. All measurements were carried out in duplicate at 22 °C.

A laboratory-assembled NSF-250 multinuclear NMR spectrometer of the Oldfield and Meadows (1978) design operating at 34-MHz ^{17}O NMR resonance frequency was used for ^{17}O NMR measurements. Both H_2O and D_2O were used as dispersion media. Single-pulse experiments were done in duplicate at 20 °C. A pulse width of 51 μs and a recycling time of 0.22 s were used. The spectra were retained in an 8K point array that provided adequate resolution.

Additional ^{1}H NMR relaxation measurements on flour dispersed in D_2O were carried out at 20 MHz with a PC-20 NMR process analyzer (IBM Instruments Corp., Danbury, CT). The CPMG and the OW multipulse sequences were generated with an EPROM (IBM Instruments, Corp., Danbury, CT), for T_2 measurements; these measurements were made in triplicate at 25 °C.

Figure 1 shows the Fourier transform spectra of a 90% wheat flour–water system. Figure 1A presents a 360-MHz ^{1}H NMR spectrum, and Figure 1B presents its corresponding 34-MHz ^{17}O spectrum. Line widths were measured at half-height of each spectrum. To correct for any residual magnetic field inhomogeneity, the net line broadening ($\Delta\nu_B$) was calculated by substracting the line width of liquid water or 0.2% HDO in D_2O ($\Delta\nu_{free}$) from that of the sample ($\Delta\nu_{obsd}$). The net or differential transverse relaxation rates (ΔR_2, s^{-1}) were then calculated from the line widths by the standard formula (1). All D_2O

$$\Delta R_2\ (s^{-1}) = \pi\Delta\nu_B\ (s^{-1}) = \Delta T_2^{-1}\ (s) \qquad (1)$$

replacements of H_2O were done on a molar basis in order

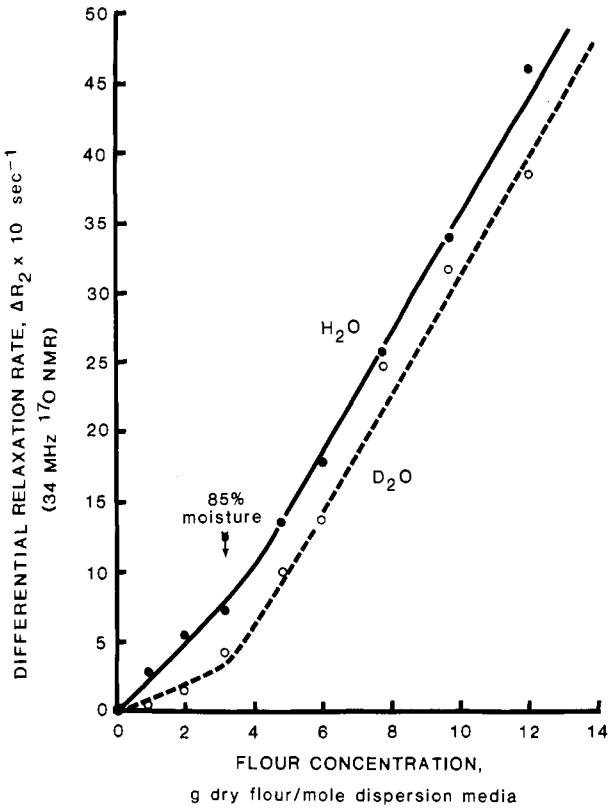

Figure 2. Dependence of the differential oxygen-17 NMR transverse relaxation rate on wheat flour concentration (dry basis) in both water and deuterium oxide dispersing media.

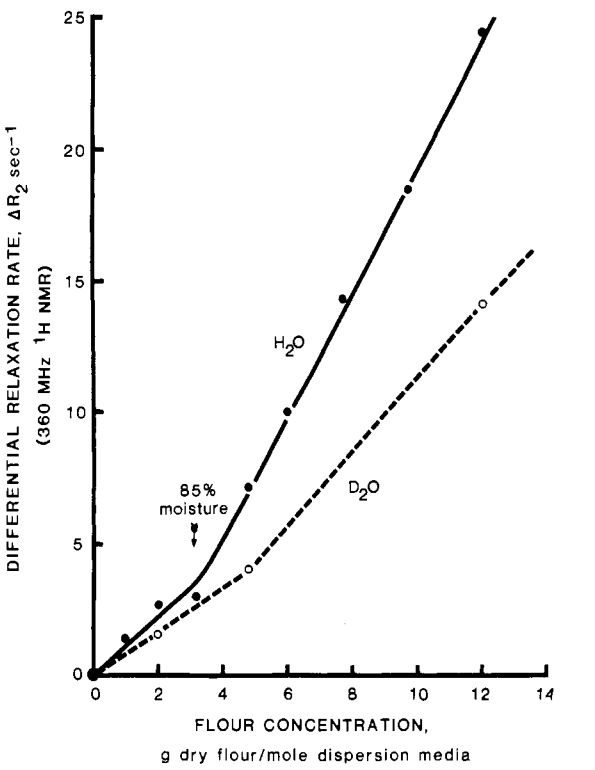

Figure 3. Dependence of the 360-MHz differential proton NMR transverse relaxation rates on flour concentration, with water or deuterium oxide as the dispersing medium, using the OW multipulse sequence.

to obtain an appropriate scaling of the molecular H_2O and D_2O interactions that are monitored by NMR. Similar data were obtained for all other concentrations of wheat flour in H_2O/D_2O.

RESULTS AND DISCUSSION

Relaxation Models. There are several different models proposed for the interpretation of the T_1 and T_2 relaxation data (Child and Pryce, 1972; Berendsen, 1975; Halle et al., 1981; Derbyshire, 1982; Finney et al., 1982; Kumsinski and Pessen, 1982; Shirley and Bryant, 1982). The isotropic two-state model with fast exchange (Zimmerman and Brittin, 1957; Derbyshire, 1982) will be specifically applied here in two interpretations. In the isotropic, two-state model with fast exchange, a linear relationship between the observed relaxation rate (R_{obsd}) and macromolecular concentration is predicted, if no additional contributions to relaxation are present. This relationship is based on a population-weighted average between "bound" (R_B) and free (R_F) water (eq 2), where P_B and P_F are the percent bound and percent free water in the system, respectively. Departures from such linearity are often observed at higher concentrations, such as those shown for the data in Figures 2 and 3.

$$R_{obsd} = P_B R_B + P_F R_F \quad (2)$$

Figure 2 presents the dependence of the ^{17}O NMR ΔR_2 on flour suspensions in H_2O and D_2O. This shows two linear regions: one from 5 to 15% flour (0.94–3.18 g of flour/mol of dispersion media) and another from 20 to 40% flour (4.8–12.0 g of flour/mol of dispersion media). Similarly, Figure 3 presents the dependence of the ^{1}H NMR ΔR_2 on flour suspensions in H_2O and D_2O. Again, this plot shows two linear regions, with a change in relaxation rate occurring at approximately 15% flour (3.18 g of flour/mol of dispersion media). Because of the observed nonlinearity, either additional water "states" must be considered or else new interactions are present that must be taken into account. Two models for this departure are proposed. The first is presented in a review by Derbyshire (1982) and the second by Kumosinski and Pessen (1982).

Derbyshire Model. Derbyshire (1982) proposed two possible explanations for this nonlinearly at higher concentrations: (1) a change in the hydration of the macromolecule; (2) a change in the relaxation rate of the bound water. In Figures 2 and 3, the increase in ΔR_2 may be ascribed to a change in the observed relaxation rate of the bound water caused by entanglement or possibly microscopic aggregation of the molecular side chains, such as those of protein in the flour, or alternatively aggregation of starch granules. The motion of the molecular side chains and the water hydrating them is decreased as the concentration is increased due to their interaction and/or entanglement with one another. This slower molecular motion will be reflected in a larger ΔR_2 for the bound water component. Assuming a fast exchange between the water of hydration at such binding sites and the "bulk" water on the NMR time scale, this larger ΔR_2 for the bound water will cause the observed relaxation rate, which is an average over "bound" and free water, to increase. Such an increase in relaxation rate would result in an increased gradient at the high concentration, as can be seen in Figures 2 and 3.

This increase in T_2 relaxation rates observed here reflects only the motion of the water of hydration of localized side chains, not the tumbling motion of entire flour particles. Brown and Pfeffer (1981) recently studied the motion of protein side chains and reported a correlation time (τ_c) of 48 ps for deuterium-labeled lysine groups. Any water "bound" to such groups should then have a τ_c value that is not longer than that of the side chain itself, 48 ps. This τ_c value is in good agreement with the τ_c values calculated from the ^{17}O NMR relaxation rates under the

Table I. Parameters Determined with Equation 4 by Nonlinear Regression Analysis of ^{17}O NMR Relaxation Rates (H_2O and D_2O) (Figure 2)

parameter	D_2O	H_2O
$n_H R_B$ (slope)	6.43 ± 3.18	15.55 ± 4.33
B_0	0.16 ± 0.05	0.06 ± 0.03
B_2	$(-1.05 \pm 0.33) \times 10^{-2}$	$(-0.26 \pm 0.17) \times 10^{-2}$
R_F (intercept)	234.5 ± 11.4	210.4 ± 8.8
RMS[a]	7.938	4.741

[a] Regression mean square.

two-state model with fast exchange as reported below.

It must be noted that the increase in ΔR_2 for localized side chain hydration water is many orders of magnitude smaller than that predicted by the Stokes–Einstein model (Atkins, 1978; Berliner and Reuben, 1980) for rotational diffusion of entire particles.

Derbyshire (1982) stated that departures from linearity usually occur at solute concentrations of the order of 20% by weight. In a study by Halle et al. (1981), this change in the relaxation rate gradient was observed between 5 and 10% in lysozyme and human plasma albumin solutions. A report by Oakes (1976) showed a marked increase in the T_2 relaxation rate at 10% native bovine serum albumin. Oakes attributed this change in the relaxation gradient to the cooperative effect of adjacent protein molecules on surrounding water. As shown in Figure 2 and 3, the transition in our wheat flour suspension was observed at approximately 15% flour.

Kumosinski Model. The second model for the observed nonlinearity of the concentration dependence of the NMR relaxation rates was proposed by Kumosinski and Pessen (1982) for dilute protein solutions. The deviations from linearity were attributed to charge repulsion or to charge fluctuations as predicted by the Kirkwood–Shumaker (1952) theory. The major application of the Kumoskinski and Pessen (1982) work to that presented here is the use of activities in place of concentrations when dealing with systems strongly deviating from ideality. The activity of a substance (a) in solution is related to its concentration by the activity coefficient γ (eq 3), where

$$a = \gamma c \tag{3}$$

c is the concentration in grams of substance/mole of water. The activity coefficient can be obtained from the virial expansion of osmotic pressure as a function of concentration according to eq 4, where the B parameters are the virial coefficients.

$$\mathrm{d} \ln \gamma / \mathrm{d}c = 2B_0 + 3B_2 c + \ldots \tag{4}$$

The Kirkwood–Shumaker theory was applied by Kumosinski (1985) to the ^{17}O NMR H_2O and D_2O relaxation data (Figure 2), with the two-state assumption yielding eq 5, where R_{obsd} is the observed NMR relaxation rate (s^{-1}),

$$R_{obsd} = n_H R_B c e^{2B_0 c + 1.5 B_2 c^2} + R_F \tag{5}$$

n_H is the Scatchard hydration (moles of bound water/grams of flour), R_B is the relaxation rate for the bound water, c is the concentration (grams of flour/mole of H_2O or D_2O), B_0 and B_2 are the second and third virial coefficients, respectively (mol/g), and R_F is the relaxation rate of the free, liquid water (s^{-1}).

According to this theory, plotting the NMR relaxation rate against "activity" of the substance (i.e., wheat flour) will yield a linear plot. The parameters of the line correspond to the parameters of eq 5; the slope is equal to $n_H R_B$, and the intercept is equal to R_F. To calculate the "activity" of the wheat flour we first need to solve eq 5 for

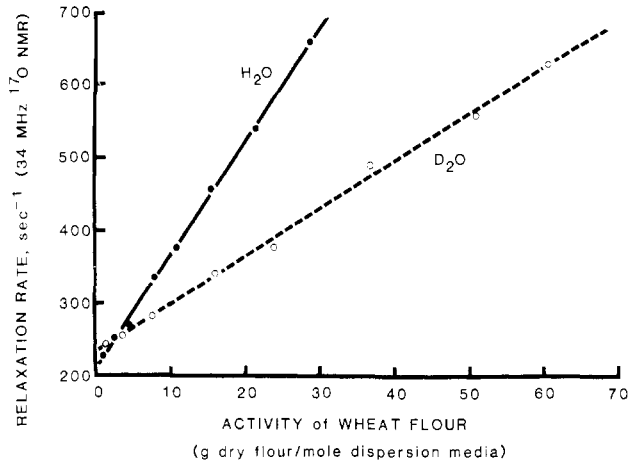

Figure 4. Linear dependence of the ^{17}O NMR transverse relaxation rate on the flour "activity" of a wheat flour suspension in water and in deuterium oxide.

the virial coefficients B_0 and B_2. ("Activity" is used in quotes because wheat flour is not soluble but only dispersible in water). This can be done by nonlinear regression analysis employing a modified Gauss–Newton computer program (Kumosinski, 1985). The resultant equation parameters and associated errors are presented in Table I. We can then use the resultant B_0 and B_2 values to solve for γ in eq 4. Finally, the "activity" is calculated from eq 3. As shown in Figure 4, a linear relationship is obtained when the ^{17}O NMR transverse relaxation rate, for both D_2O and H_2O, is plotted against the calculated wheat flour "activity" (correlation coefficient 0.998 for D_2O and 0.999 for H_2O). Two concerns are to be noted: (1) the relatively large magnitude of the errors associated with some of the parameters of eq 5; (2) the large difference between the D_2O and H_2O results. The first concern reflects the need to further test the proposed model with a variety of systems. As for the second concern, this difference may be due to the proton-exchange line broadening that occurs in H_2O but not in D_2O. This is evidenced by the higher slope of the H_2O line in Figure 4.

The Kumosinski model may account for the nonlinearity of the NMR relaxation data (Figure 2) by attributing the change in the gradient of the T_2 relaxation rate as a function of concentration to the attraction of the flour particles by charges on the molecular components of the flour (Marsh et al., 1982). Further investigation of the transition at 15% flour will be necessary to establish which of the two models is the most appropriate.

Proton Exchange. Another aspect of Figures 2 and 3 to be considered is the difference in the relaxation rates between the H_2O and D_2O suspensions. The difference between the ^{17}O transverse relaxation rates of H_2O and D_2O flour suspensions (Figure 2) is likely to be caused by the presence of proton-exchange broadening of the ^{17}O NMR resonance (Meiboom and Gill, 1958; Halle et al., 1981). The D_2O practically eliminates the proton-exchange broadening of the ^{17}O NMR line width and thus leaves the quadrupolar relaxation as the dominant broadening mechanism (Dwek, 1973; Laszlo, 1983). The ^1H NMR transverse relaxation rates (Figure 3) were also substantially decreased for the D_2O samples over the H_2O samples, suggesting that proton exchange is a significant source of transverse relaxation in the wheat flour suspensions.

Dough Concentration Range. Figure 5 presents the 360-MHz ^1H NMR transverse relaxation data for the extended range of flour concentrations (95–30% moisture) (0.94–42.1 g of dry flour/mol of H_2O). This shows two

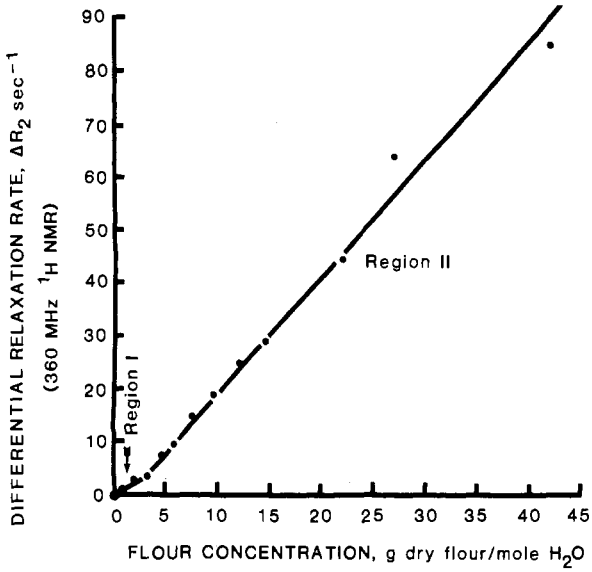

Figure 5. Dependence of the 360-MHz proton NMR differential relaxation rate on flour concentration extended from Figure 3 to the full range of flour concentrations (95–30% moisture).

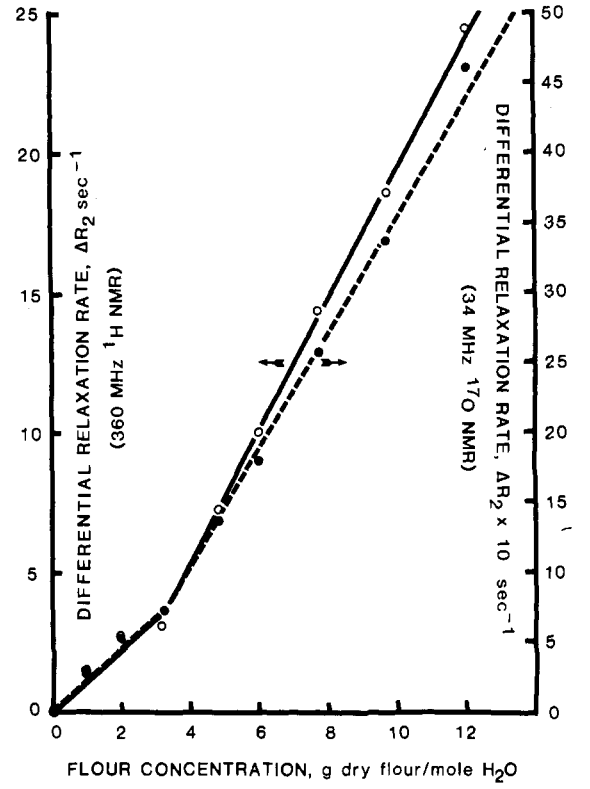

Figure 6. Dependence of 34-MHz oxygen-17 and 360-MHz proton NMR differential relaxation rates on wheat flour–water concentration over the range 95–60% moisture.

linear regions. Region II remained linear throughout the dough range, even up to 70% flour (42.1 g of dry flour/mol of H_2O). This is indeed surprising when one recalls the extensive rheological changes in the flour–water system as the concentration of flour is increased from a "liquid" suspension to a semisolid dough (Richardson, et al. 1985a). Figure 5 shows that the T_2 of water decreased linearly with increasing flour concentration over this range. This indicates that, for a unit weight of dry flour, the product of ΔR_2 and number of moles of water remains constant over region II.

Comparison of Nuclei. Figure 6 is a comparison between the ^{1}H and ^{17}O NMR relaxation rate of flour suspensions in water. As expected, the two nuclei yield different absolute relaxation rates due to the different relaxation mechanisms (Derbyshire, 1982). However, the similarity in the concentration dependence measured by the ^{1}H and the ^{17}O NMR strongly suggests that the proton NMR transverse relaxation rates are primarily monitoring the binding of water molecules to the flour. Lioutas et al. (1985b) drew similar conclusions from ^{1}H, ^{2}H, and ^{17}O NMR studies of hydrated lysozyme, as did Tricot and Niederberger (1979) in their study of dipalmitoyl-3-*sn*-phosphatidycholine (DPL). Koenig et al. (1975) also compared the field dispersion of the relaxation rates of solvent ^{1}H, ^{2}H, and ^{17}O in aqueous solutions of lysozyme. These authors observed that the field dispersion of relaxation rates of the three solvent nuclei, normalized to their respective rates in pure water, were essentially the same. They concluded that it is the entire solvent water molecules, not the exchange of protons, that are involved in the interaction.

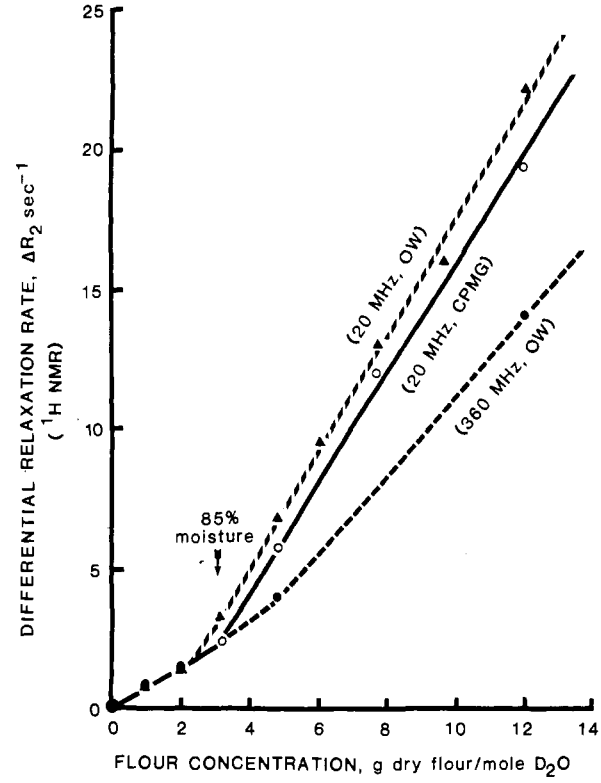

Figure 7. Dependence of the differential proton NMR relaxation rates on flour concentration in D_2O at two frequencies and two multipulse sequences (OW and CPMG).

Pulse Sequence Dependence. Figure 7 shows the dependence of two multipulse sequences, CPMG (Meiboom and Gill, 1958) and OW (Ostroff and Waugh, 1966), on flour concentration at 20-MHz proton NMR resonance frequency.

The general trend of two linear regions and a transition at approximately 85% moisture (15% flour) as found above is apparent with both multipulse sequences (Figure 7). Both CPMG and OW multipulse sequences give comparable ΔR_2 values in the "liquid" range. However, in the case of high-viscosity liquids or semisolids, the CPMG sequence may be less useful because strong dipolar interactions can dominate the spin echo behavior (Baianu et al., 1978, 1985). The OW sequence has the advantage that it can be used over the entire concentration range from suspensions to dough systems (Figure 5). This sequence effectively eliminates almost all experimental broadening factors, e.g. magnetic field and sample inho-

mogenities and dipolar interactions.

Frequency Dependence. The dependence of the proton ΔR_2 on magnetic field strength (Figure 7) was explored by comparing ^1H NMR relaxation data at 20 and 360 MHz with the OW multipulse sequence at several flour concentrations. The 20- and 360-MHz ^1H NMR data at the low flour concentrations were virtually the same. This indicates that the NMR relaxation conditions are in the extreme narrowing limit. Therefore, we can use the ^{17}O NMR data to calculate the correlation time, τ_c, of the water "bound" in wheat flour. The difference between the data at 20- and 360-MHz ^1H NMR frequencies at the high flour concentration (Figure 7) is likely to be caused, at least in part, by cross-relaxation (Kalk and Berendsen, 1976; Edzes and Samulski, 1978). This difference is not, however, as large as would be expected if cross-relaxation was the dominant NMR relaxation mechanism.

Correlation Time. In the limit of fast exchange when the dominant relaxation mechanism is the interaction of the quadrupole moment of the ^{17}O nucleus with the fluctuating electric field gradients at the nucleus, τ_c can be calculated (Dwek, 1973; Halle et al., 1981) from eq 6, where

$$\tau_c = \Delta R_{2B}[K(e^2qQ)^2(1 + (\eta^2/3))]^{-1} \quad (6)$$

τ_c is the correlation time for rotation, or reorientation, of the water molecules in seconds, $K = 12\pi^2/125$, e^2qQ is the quadrupole coupling constant (Abragam, 1961) in Hertz, ΔR_{2B} is the transverse relaxation rate of the bound water component, and η is the asymmetry parameter. The calculation of τ_c was given in detail by Lioutas et al. (1985b), as explained by Dwek (1973). To calculate τ_c from eq 6, we need ΔR_{2B}, e^2qQ, and η. On the basis of the two-state model, ΔR_{2B} is the slope of the plot of ΔR_2 against the "bound" water fraction (moles of H_2O bound/mole of H_2O total). To calculate this fraction requires a value for the amount of water "bound" by a specific system; this was calculated to be 18.8 g of water/100 g of dry matter from the sorption isotherm of wheat flour (Iglesias and Chirife, 1982), using the graphical determination of the D'Arcy and Watt sorption isotherm parameters (Richardson et al., 1985b). ΔR_{2B} was calculated from this analysis to be 907.2 s^{-1}.

Two values of the quadrupole coupling constant ($I = e^2qQ$) for the bound water were previously suggested (Lioutas et al., 1985b): $I_h = 6.67$ MHz, as in ice (with $\eta = 0.93$), and $I = 4.03$ MHz (in the presence of a small anisotropy with the order parameter, $s = 0.06$). With these values, τ_c values for the bound water molecules in the wheat flour were calculated to be 16.7 ps (in the case of $I_h = 6.67$ MHz) or 45.8 ps (in the case of $I = 4.03$ MHz). These values are useful first estimates of the molecular mobility of the bound water in the flour system. These τ_c values are between 5.5 and 14.5 times greater than that of free D_2O, which has a τ_c value of 3.1 ps (Halle et al., 1981). The fast motion of the bound water suggests that the molecular motion of bound water and/or local molecular motion of the binding sites is much greater than previously hypothesized (Oakes, 1976; Fung and McGaughy, 1979) and is in good agreement with recent ^{17}O NMR work on lysozyme (Lioutas et al., 1985b) and other proteins (Halle et al., 1981; Laszlo, 1983).

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Evaluation of Foliar Application and Stem Injection as Techniques for Intrinsically Labeling Wheat with Copper-65

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Foliar application and stem injection were evaluated as techniques for intrinsically labeling wheat with ^{65}Cu. The copper concentrations in the whole wheat flours were as follows: 9.1 $\mu g/g$, control; 12.9 $\mu g/g$, foliar application; 22.1 $\mu g/g$, stem injection. Four protein fractions (globulins and albumins, glutenins, gliadins, remaining proteins) were sequentially extracted from defatted whole wheat flours. Gel column chromatography of the fractions demonstrated that the elevated concentrations of copper in the foliar-applied and stem-injected wheat did not alter any of the significant proteins based on V_e/V_0 values. These results indicate that the bioavailability of copper should not differ among the control and the ^{65}Cu-treated wheats.

INTRODUCTION

Copper is widely distributed in foodstuffs, but information on the proportion of dietary copper actually absorbed is limited. A daily copper intake of 2–3 mg is recommended for adults (National Academy of Sciences, 1980), but dietary surveys show that actual intake is often below 1 mg/day (Klevay, 1975). Absorption of copper as $CuCl_2$ from purified diets (Turnlund et al., 1982; Turnland, 1984; King et al., 1978) and in the fasting state (Johnson, 1984) has been studied in humans, but the bioavailability of copper to humans from foodstuffs is virtually unknown. With the increased emphasis on determining the bioavailability of trace elements from major foodstuffs, intrinsic labeling of foods with isotopes has become essential.

Previous work in this laboratory demonstrated that sufficient enrichment of wheat with ^{65}Cu to be used in human bioavailability studies can be obtained by stem injection or foliar application of ^{65}Cu (Lykken, 1984). But the question of whether these techniques altered the distribution of Cu or the protein composition of wheat remained unanswered. This study was initiated to evaluate foliar application and stem injection of ^{65}Cu as techniques for intrinsically labeling wheat using Cu distribution and the composition of various protein fractions as the major criterion for comparison.

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METHODS AND MATERIALS

Wheat, *Triticum aestivum* var. Waldron, was grown in a greenhouse with supplemental lighting provided by 400-W high-pressure sodium lamps (Energy Technics, York, PA) to produce a 16-h light/8-h dark cycle. Plants were grown in 8-in. plastic pots, seven plants per pot, in soil with 30% perlite. A total of 70 plants were grown for each of the control and foliar-application and stem-injection treatments. Plants were watered and fertilized (20:20:20) (Peter's Fertilizer, W. R. Grace and Co., Fogelsville, PA) as needed.

Since copper has only two short-lived radioisotopes, ^{64}Cu ($t_{1/2}$ = 12.0 h) and ^{67}Cu ($t_{1/2}$ = 61.9 h), only the stable isotope, ^{65}Cu, is suitable for studies of copper absorption in humans. Thus, ^{65}Cu as $^{65}CuCl_2$ (Oak Ridge National Laboratory, Oak Ridge, TN) was applied to wheat plants as outlined below. Foliar application of ^{65}Cu during anthesis was performed as follows: $^{65}CuCl_2$ was added to a citrate–phosphate buffer (McIlvaine, 1921), pH 5.5, plus 1% sodium lauryl sulfate. The final concentration of ^{65}Cu was 0.3 mg/0.7 mL of solution. This solution (0.7 mL/plant) was sprayed onto the plants with a hand-held plant sprayer. A published method was used for stem injections of $^{65}CuCl_2$ at a rate of 0.3 mg of ^{65}Cu/plant (Starks and Johnson, 1985). In this study 0.2 mL of the solution was injected compared to the 0.7 mL previously reported