^{17}O Nuclear Magnetic Resonance Studies of Water Mobility during Bread Staling

Mal-Shick Kim-Shin,† Frank Mari,‡ Pramila A. Rao,† Thomas R. Stengle,‡ and Pavinee Chinachoti*,†

Departments of Food Science and Chemistry, University of Massachusetts, Amherst, Massachusetts 01003

The ^{17}O signal from bread treated with enriched water was observed during staling. Bread samples containing various humectants and surfactants (as well as a control sample containing neither) were used. Both the transverse relaxation time (T_2) and the integrated signal intensity were monitored. During staling T_2 decreased by 20–30%, and the intensity decreased by 0–22% depending on the nature of the additives. The NMR data do not correlate with the inhibition of amylopectin crystallization by surfactants. We conclude that the high-resolution NMR experiment is sensitive to only that fraction of water which is present in the amorphous region. The changes in T_2 and intensity were due to changes which took place in that region.

INTRODUCTION

Back in 1902, starch retrogradation, the recrystallization of starch, was proposed to be the predominant factor in the staling of bread (Lindet, 1902). Now, after nearly a century of research, starch retrogradation is still believed to be a major contributor to the staling process (Schoch and French, 1947; D'Appolonia and Morad, 1981; Maga, 1975; Kulp and Ponte, 1981; Krog et al., 1989). However, additional information has been gathered which indicates that staling involves a combination of related mechanisms, rather than the starch component alone. Some of these include changes in the gluten functionality (Maga, 1975; Kulp and Ponte, 1981), moisture migration/redistribution (Leung, 1981; Leung et al., 1983; Cesari, 1974), amylose (Kim and D'Appolonia, 1977), and, recently, the glassy/rubbery state of bread polymers (Slade and Levine, 1990).

Today many workers believe that the recrystallization of amylopectin is aided by water-mediated plasticization (Lechert, 1981; Wynne-Jones and Blanshard, 1986). Willhoft (1973) proposed that this water is released from the denatured gluten which slowly decreases its "water holding capacity". He claimed that up to 30% of the moisture associated with the gluten fraction migrated to the starch in 120 h of room temperature storage. However, other workers suggested that the water transfer takes place in the opposite direction, from starch to gluten (Cluskey et al., 1959; Zobel, 1973). Recently there has been considerable interest in the dynamic state of water in bread, and the role of "bound" and "free" water is thought to be an important factor in the staling process (Kulp and Ponte, 1981).

A number of workers have utilized nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) methods to study the state of water in bread. Leung et al. (1983) applied deuterium (^2H) NMR to bread staling and found that T_2 decreased over time. This is consistent with the earlier proposal of Leung (1981) that "as bread stales, starch changes from the amorphous state to the more stable crystalline state, which immobilizes the water molecules by incorporating them into the crystalline structure". Wynne-Jones and Blanshard (1986) used DSC to ascertain the state of water in aging starch gels. They found a significant increase in unfreezable water with time. They also observed the relaxation time, T_2, of the water proton, and they concluded that the change in the physical state of the water occurred primarily in the amylopectin fraction. Recently, Slade and Levine (1990) performed a DSC study of staled bread and suggested that the freezable water in the amorphous matrix migrated to the crystalline hydrate of B-type wheat starch. Here the water became unfreezable.

It has been shown that studying water in biological systems by ^1H and ^2H NMR is subject to error due to (1) cross-relaxation between protons of the water and the matrix and (2) exchange of ^2H on water with ^1H of the matrix (Edzes and Samulski, 1978; Shirley and Bryant, 1982; Richardson and Steinberg, 1987; Kakalis and Baianu, 1988; Mora-Gutierrez and Baianu, 1990). It is not possible to estimate the effect of these phenomena, and ^{17}O NMR must be used if reliable results are to be obtained (Richardson and Steinberg, 1987; Kakalis and Baianu, 1988; Mora-Gutierrez and Baianu, 1990). Recent work has shown that a combination of relaxation time and integrated signal intensity measurements on ^{17}O can yield valuable information regarding the water mobility in a food model system (Chinachoti and Stengle, 1990).

In this work we have applied ^{17}O NMR to study the changes in water mobility during bread staling. We compared the behavior of surfactant-treated bread with that of a control sample (no surfactants added). A comparison of the two could give information about the role of the surfactant in amylopectin crystallization.

MATERIALS AND METHODS

Bread. Bread doughs were made from untreated bread flour (King Arthur Flour Co., Andover, MA). According to the manufacturer, the flour contained 12.2% protein, 0.49% ash, and 13.1% water. The surfactants used were sodium stearoyl-2-lactylate (SSL) (Patco Products, Kansas City, MO), mono- and diglycerides (Dimodan, Grinsted Products, Inc., Kansas City, MO), and sucrose ester (SE 1170, Ryoto Augar Ester, Mitsubishi-Kasei Food Corp., Tokyo, Japan). Humectants (glycerol and sorbitol), calcium propionate, and potassium sorbate were of analytical grade (Fisher Scientific Co., Fair Lawn, NJ). Sodium chloride, sucrose, nonfat dry milk, active dry yeast, and shortening (Crisco, Proctor and Gamble, Cincinnati, OH) were obtained locally.

Nine types of bread were made: a control containing no humectants or surfactants, four sorbitol-containing breads with

* Address correspondence to this author.
† Department of Food Science.
‡ Department of Chemistry.

0021-8561/91/1439-1915$02.50/0

Table I. Straight Dough Formula[a]

ingredient	formula, g	ingredient	formula, g
flour	803.7	salt	12.1
water	482.2	calcium propionate	5.0
sugar	37.8	potassium sorbate	3.0
active dry yeast	16.1	surfactant (if applied)	4.0
nonfat dry milk	16.1	humectant (if applied)	40.2
shortening	24.1		

[a] Batch size: 1400.1 g with no surfactant and humectant; 1404.1 g with surfactant; 1440.3 g with humectant; 1444.3 g with both. Mix time: 10 min (Hobart Model N-50 mixer) at speed 2. Dough temperature 32 °C; floor time 15 min; intermediate proof 30 °C, 80% RH, 10 min; 500 g of dough was divided and molded manually, proofed at 30 °C, 80% RH, for 1 h or until height reached top of pan, baked 20 min at 193.3 °C (Garland Model 680-Z electric oven, Garland Commercial Industries, Inc., Freeland, PA).

four surfactant treatments, and four glycerol-containing breads with four surfactant treatments. The four surfactant treatments were no surfactant, Dimodan, SSL, and sucrose ester.

Bread Baking. The bread was made using a straight dough formula and procedure (Pyler, 1988) as shown in Table I. Part of the water (50 mL) was tempered to 32 °C (dough temperature) and suspended with the yeast. The balance of the water was mixed with sugar, nonfat dry milk, calcium propionate, potassium sorbate, and salt for 2 min. Half of the flour was added and mixed for 30 s, and then yeast and the rest of the flour were added. After an additional 2 min of mixing, the shortening (with surfactants and humectants, if applied) was mixed in. Mixing of the dough was continued until the total mixing time was 10 min. After proof, two 500-g portions were molded manually into loaves, proofed for 1 h, or until the height reached the top of the pan, and then baked for 20 min at 193.3 °F. Additional conditions and parameters are given in Table I.

Sample Preparation. After baking, the bread was cooled to room temperature (2 h) and sliced into eight 1-in. slices. Slices 2, 3, 6, and 7 were selected for study. From each of these four slices, a cylinder of 30-mm diameter was taken out with a cork borer from the center of the slice.

The moisture content of the bread crumb was determined according to the AOAC (1984) vacuum oven method, and water activity (a_w) was measured by an isopiestic method (McCune et al., 1981). The moisture contents of the bread crumb were 0.557 ± 0.012, 0.537 ± 0.007, and 0.513 ± 0.008 g of water/g of solids for the control, sorbitol-treated, and glycerol-treated bread, respectively. There were no significant differences in moisture content among the various surfactant treatments for breads with the same humectant treatment. The value of a_w for the humectant-free fresh bread was 0.97, and that for all humectant-treated fresh breads was 0.93.

The control bread samples were adjusted to various moisture contents using saturated salt solutions containing water enriched to 1.0–3.3% oxygen-17. This was done by absorption or desorption in three a_w-controlled chambers [0.88, 0.93, and 0.97 a_w at 25 °C using saturated solutions of zinc sulfate, potassium nitrate, and potassium sulfate, respectively (Greenspan, 1977)].

All eight treated bread samples were also enriched in oxygen-17 by placing a small portion of the cylinder above a saturated solution of potassium nitrate made up with oxygen-17 enriched water to achieve an a_w of 0.93 (Greenspan, 1977). The bread was left in the 0.93 a_w chamber for 12 h at 25 °C, after which time weighed samples were placed in NMR tubes and sealed. There was some unavoidable staling during the time required for enrichment and other necessary delays prior to the first NMR measurement. Therefore, the first measurement was made 24 h after baking for all samples. This point is called day 1. The samples were kept at room temperature throughout the study. In some cases moisture content was determined before and after the aging study. At most the humectant-treated samples lost 2% of their moisture. Since the samples were sealed with tight-fitting polyethylene caps during aging, this loss was limited to evaporation into the head space and losses during handling.

NMR Measurements. The ^{17}O NMR spectra at 40.67 MHz were recorded on a Varian XL-300 broad band spectrometer retrofitted with a VXR-4000 computer system. A spectral width of 20 000 Hz and a 15-μs pulse (corresponding to a flip angle of 79°) were used to obtain the spectra. Maximum sensitivity was obtained by using a short acquisition time (0.02 s) and a large number of repetitive accumulations (4000–60 000). Acoustic ringing was found to distort the base line in most samples; hence, to avoid this problem, a 150-μs delay (dead time) between the pulse and the acquisition was introduced to suppress the "rolling base line effect" (Brevard, 1983). The introduction of the dead time did not have a significant effect on the line width and the shape of the NMR spectra; however, it did simplify the line-width measurements. Proton decoupling was used during acquisition, but the samples were not spun. In fact, neither of these was found to have any significant effect on the spectra.

Table II. ^{17}O NMR Transverse Relaxation Times (T_2) for Sorbitol-Treated Breads with Four Surfactant Treatments[a]

	transverse relaxation time, (T_2), ms	
bread	200-AF	XL-300
standard (no surfactant)	0.259	0.253
with SSL	0.241	0.239
with SE	0.220	0.233
with Dimodan	0.245	0.241

[a] All samples were obtained from day 1 of storage. Spectra were obtained at 20 °C using both IBM 200-AF and Varian XL-300 spectrometers.

It is difficult to observe signals with relaxation times less than 150 μs with the Varian XL-300, and there is a possibility that a broad component of the spectrum may be lost. Consequently, some of the samples were submitted to ^{17}O NMR analysis at 28.7 MHz using an IBM 200-AF spectrometer with IBM Solids Accessory. This instrument has a higher radio-frequency power for spin excitation and a different probe design. It allows for a dead time of ca. 15 μs. Using similar acquisition times and numbers of accumulations, there were no large differences in the T_2 values obtained on both instruments, the largest being 6% (Table II). Magic angle spinning (MAS) had no effect on the ^{17}O spectrum. The signal from every sample was a simple Lorentzian line, regardless of which instrument was used.

Since no signals were observed in the range 15 μs < T_2 < 150 μs, the water in the bread samples falls into two distinct categories. That with T_2 > 150 μs corresponds to "mobile" water, and it is readily observed with the Varian XL-300. The fraction with T_2 < 15 μs cannot be detected with either instrument. As an operational definition, we take this to be "bound" water.

^{17}O NMR Spectra. All of the results shown in Figures 3–5 were obtained with the Varian XL-300 instrument. A single Lorentzian line was obtained in all cases. The transverse relaxation time was obtained by measuring the line width of the ^{17}O peak in the NMR spectra and using the standard formula for T_2

$$T_2 = 1/R_2 = 1/(\pi\Delta\nu)$$

where R_2 is the transverse relaxation rate and $\Delta\nu$ is the line width at half-height.

The amount of detectable water was monitored using the absolute integrated signal of the NMR spectra (Chinachoti and Stengle, 1990). The volume of the samples was always less than 1.2 mL (the maximum effective volume observable with the 10-mm NMR probe used for the experiments). Liquid water was used as an external standard for all measurements. The ^{17}O intensities are self-consistent for each sample. They give a valid comparison of detectable water as each sample ages. However, various samples may contain different amounts of bread, as well as variable degrees of ^{17}O enrichment. Thus, it is not possible to compare the amount of detectable water from one sample to another. For this reason in Figure 5 the amount of detectable water is reported as a percentage of the day 1 value for each individual sample and not as an absolute value. The parameters define detectable water as that fraction which relaxes slower than ca. 150 μs, a figure which is determined by the dead time.

Duplicate measurements of T_2 on a single sample showed an average variation of 2%. Duplicate samples were examined in some cases. Here the average variation was 4%, which reflects small differences in sample preparation and handling. Data from

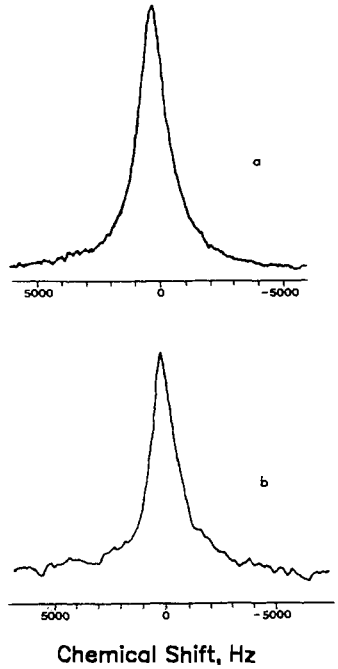

Figure 1. Fourier transformed ^{17}O NMR spectra for ^{17}O-enriched bread (sorbitol treated, no surfactant), measured under (a) the high-field Varian XL-300 and (b) the solid-state IBM 200-AF spectrometers.

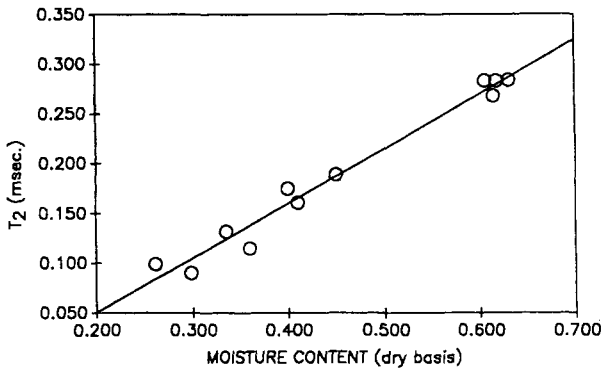

Figure 2. ^{17}O NMR relaxation time (T_2) for water in a control (humectant and surfactant free) bread of various moisture contents (obtained by desorption) showing a linear relationship (r = 0.988).

two different instruments (same samples) are shown in Table II. The average variation is 2.6% with the worst case being 5.8%.

DSC Measurements. To observe the degree of amylopectin crystallization during bread staling, the degree of endothermic melting of the crystal in several samples was measured over storage time. The samples were bread with four surfactant treatments (control, SSL added, SE added, and Dimodan added), none of which contained humectants. The crumb of these samples (10 g) was stored in sealed cans at room temperature (22 °C). The endotherms were obtained using a Perkin-Elmer DSC (DSC2, Perkin-Elmer Corp., Norwalk, CT). A 10-mg bread sample was weighed and placed in a hermetically sealed pan and heated from 7 to 160 °C at a rate of 10 °C/min. An empty pan was used as the reference. The degree of amylopectin crystallization was measured from the endothermic peak area at ca. 70 °C (commonly known as the melting endotherm for amylopectin; Krog et al., 1989).

RESULTS

Untreated Bread. The T_2 of a sample of untreated bread (no added surfactants or humectants) is plotted against moisture content in Figure 2. Within the moisture range studied (0.25–0.65 g of water/g of solids), T_2 increased linearly with moisture content (r = 0.988).

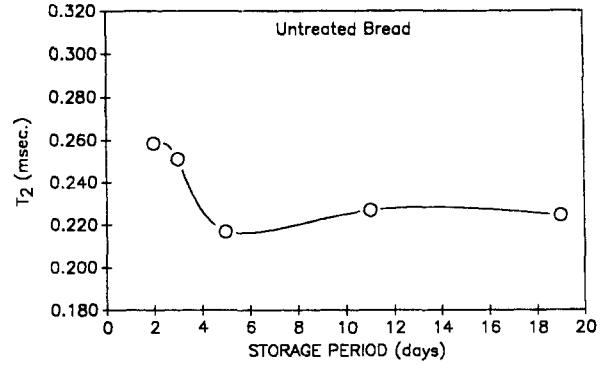

Figure 3. Changes in ^{17}O NMR relaxation time (T_2) for water in the control (humectant and surfactant free) bread during storage at room temperature.

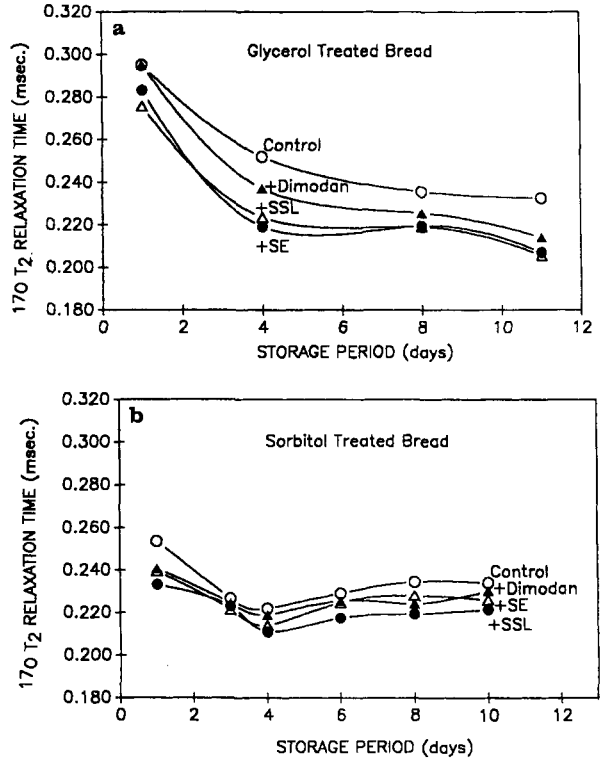

Figure 4. Changes in ^{17}O NMR relaxation time (T_2) for water in breads during storage. Breads were treated with 5% [flour basis (fb)] glycerol (a) and 5% (fb) sorbitol (b). The surfactant treatments (0.5%, fb) include Dimodan, sodium stearoyl-2-lactylate (SSL), and sucrose esters (SE).

Figure 3 shows the change in T_2 of the water in untreated bread over time. The T_2 decreased rapidly during the first 3–4 days of storage and remained relatively constant at ca. 0.22 ms afterward up to 19 days of storage. The sharp decrease in T_2 early in storage is presumed to be due to physicochemical processes accompanying staling (see Discussion).

Effects of Humectants and Surfactants. Breads made from eight formulas, two humectants times four surfactant treatments, were studied. The ^{17}O NMR T_2 relaxation time was observed over an 11-day storage period at room temperature. Plots of T_2 vs storage time are shown in parts a and b of Figure 4 for glycerol- and sorbitol-treated breads, respectively. The integrated signal intensities are shown in Figure 5.

Humectants. All breads showed a decrease in water mobility (as reflected in T_2) over the storage period as shown in Figure 4. However, the two humectants did affect T_2 differently; the glycerol-treated sample showed a significantly higher T_2 at day 1 as compared with the bread

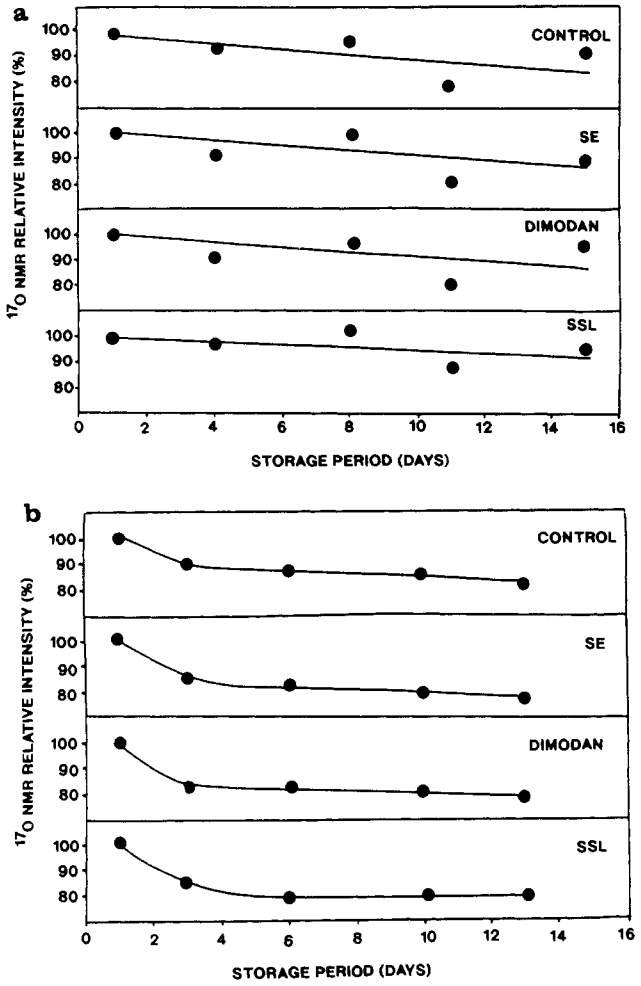

Figure 5. ^{17}O NMR relative intensity as plotted against storage time for breads treated with glycerol (a) and sorbitol (b) comparing various surfactant treatments.

treated with sorbitol, for all surfactant treatments. Over the full storage period, T_2 for both cases decreased to nearly the same level (0.22–0.24 ms) at day 11.

Figure 5 shows the change in the amount of NMR-detectable water (i.e., integrated signal intensity) in the bread samples with time. In all cases the signal intensity decreased or remained unchanged. The glycerol-treated samples (Figure 5a) showed very little loss over the 15-day period; indeed, the loss is negligible within experimental error. The sorbitol-treated samples (Figure 5b) showed a real loss of signal, and the loss is nearly the same for all surfactants. All of the samples lost ca. 22% of the signal intensity during the first 4–6 days, and it remained essentially constant thereafter. Since the samples were sealed throughout the experiment, the loss could not be due to simple evaporation beyond a small amount of moisture lost to the headspace. In interpreting Figure 5 it should be noted that the actual amount of water in each sample is not known (see Materials and Methods). Therefore, the intensity data for each sample are given as a percentage of the day 1 intensity for that particular sample.

Surfactants. Bread samples untreated and treated with 0.5% (flour basis) monoglycerides (Dimodan, SSL, and sucrose ester) all showed a decrease in T_2 on aging. In glycerol-treated bread (Figure 4a), the water mobility as determined by T_2 appears to have the following trend: no surfactant > Dimodan > sucrose ester > SSL, although the differences are not great. On aging, a sharp drop in T_2 occurred over the first 4 days, the sucrose ester treated and SSL-treated samples showing the larger drops. In sorbitol-treated bread, the T_2 behavior was quite different. The effect of the surfactants was less pronounced (Figure 4b); the initial values were lower, and the change with aging was much less. In this case there was a ca. 10% drop in T_2 over the first 4 days followed by a slight increase. The trend in T_2 is similar to that in glycerol-treated bread, but it is much less marked. At the end of 10 days, the final T_2 values were similar for both humectants, with the glycerol samples being slightly lower.

Varying the surfactant had very little effect on the behavior of the signal intensity with time. Figure 5a shows that there is essentially no change in signal intensity with time for all of the glycerol-treated samples whether they contained a surfactant or not. The sorbitol-treated samples (Figure 5b) did exhibit a drop in intensity during the first 4–6 days of storage, but the curves for all four samples have the same shape within experimental error.

Table III. DSC Endothermic Enthalpy of Melting of Crystalline Amylopectin for Breads Stored at 22 °C for 7 Days

sample	enthalpy (ΔH) cal/g of sample	cal/g of starch
control	0.227 ± 0.015	0.600 ± 0.024
surfactant treated		
Dimodan	0.180 ± 0.012	0.480 ± 0.023
SSL	0.170 ± 0.027	0.449 ± 0.057
sucrose ester	0.160 ± 0.028	0.443 ± 0.047
humectant treated		
glycerol	0.205 ± 0.021	0.455 ± 0.046
sorbital	0.153 ± 0.021	0.347 ± 0.046

Amylopectin Crystallization. The DSC results for bread treated with surfactants and humectants are shown in Table III. The enthalpy of melting shows that added surfactants had some inhibitory effect on the amylopectin crystallization as expected. The data shown here, 7 days after baking, support the report by others (Krog et al., 1989) previously suggesting that amylopectin crystallization plays a key role in bread staling.

DISCUSSION

Our results on untreated bread (no humectant or surfactant) agree with the earlier findings of Leung et al. (1983), who made a similar study utilizing deuterium NMR. They noted that in unaged bread the water mobility increased with water content, and they ascribed this to an increase in the mobile fraction of water. They also observed a decrease in the ^{2}H T_2 on aging of the bread, similar to our results (Figure 3). Some of this decrease could arise from a loss of moisture during storage; however, Leung et al. (1983) argued that the major part of the effect could not be caused by a loss of a few percent moisture. Our untreated bread samples lost <5% of their moisture. According to the data in Figure 2, a 5% moisture loss would cause a change of 0.015 ms in T_2 on average. This is about one-third of the total change in T_2 on staling. This is consistent with the observation of Leung et al. (1983), who noted that in their samples "the decrease in moisture content would account for less that 40% of the reduction in relaxation time". Our breads with humectants added showed even less than 2% moisture loss, which supports this conclusion. It has been proposed by Leung et al. (1983) and later by others (Wynne-Jones and Blanshard, 1986; Slade and Levine, 1990) that the decrease in water mobility was due to the incorporation of the water molecules (probably released from gluten upon stabilizing) into the starch crystalline structure that developed upon staling.

The T_2 values observed in this work range from 0.200 to 0.300 ms. These values are indicative of water that is less mobile than the pure liquid but which is not tightly held in a crystal lattice. The correlation time for molecular rotation of a water molecule in the liquid is 2.5×10^{-12} s (Glasel, 1972), while the T_2 values observed here lead to an average time of 70×10^{-12} s. The water of crystallization of amylopectin should be essentially rigid. It would not be detected by our spectrometer, because of the extreme quadrupolar broadening of ^{17}O in rigid solids (Cohen and Reif, 1957). Therefore, we conclude that the decrease in T_2 shown in Figure 4 is mainly due to water not incorporated in the amylopectin crystals. The water of crystallization is firmly bound, and it does not exchange with the observed water rapidly enough to affect its NMR spectrum.

It should be noted that there could have been some water in the amorphous regions that was so "tightly bound" that it did not exchange rapidly with other water. Thus, during the process of ^{17}O enrichment, this water was not exchanged, and thus it was not detected due to (1) its low ^{17}O population and (2) its very low T_2.

Since only mobile water can be observed in the NMR experiment, it is possible to ascertain whether any of this water became bound during the staling such that its signal could not be observed. This process would show up as a drop in the integrated signal intensity during staling. For the most part, the loss of intensity was small. Within experimental error, varying the surfactant had no effect on the degree of signal loss. Even the samples containing no surfactant did not differ significantly from the others. This is surprising since surfactants are known to retard bread staling, which is believed to be greatly influenced by the degree of water "binding" (Bushuk and Mehrota, 1977a,b). Moreover, an independent study in this laboratory demonstrated that surfactants inhibit the amylopectin crystallization during staling (Rao, 1991). In this work DSC was used to measure the amount of crystallized amylopectin after 7 days of aging. The relevant data are presented in Table III. Similar results have also been obtained by Krog et al. (1989).

If the drop in T_2 and signal intensity during storage were due to amylopectin crystallization, the samples with less crystallization (i.e., samples with added surfactant) should have shown a smaller decrease in T_2 and/or signal intensity as compared to samples without surfactant. However, this was not the case; within experimental error, the surfactant-treated samples behaved the same as the controls which contained humectant only. Consequently, the redistribution of water during staling is most likely related to the amorphous regions (amylopectin, gluten, and amylose) and/or the crystalline amylose. However, amylose crystallizes immediately after baking, and thus it does not play a role in the staling process (Schoch and French, 1947; Maga, 1975).

There is a curious difference in behavior between the glycerol- and sorbitol-treated samples. The glycerol-treated bread showed a larger drop in T_2, while the sorbitol-treated material had the larger drop in signal intensity (Figures 4 and 5). The cause of this inverse relationship cannot be known until we have a better understanding of the processes taking place in the amorphous region during staling.

CONCLUSIONS

The water we observed is a mobile fraction which is presumably in an amorphous region. The T_2 decreased by 20–30% during staling. This could not have been caused by moisture loss, since the samples were sealed. Furthermore, it could have not been caused by fast exchange with water of crystallization; otherwise, there would have been a much larger drop in T_2 during the crystallization process.

We found no correlation between the amylopectin crystallization and either T_2 or signal intensity during staling. Addition of antistaling surfactants, which inhibit amylopectin crystallization, did not significantly affect either T_2 or the signal intensity. Therefore, we propose that the effects observed here are not caused by amylopectin crystallization but are more likely due to changes that take place within the amorphous region.

ACKNOWLEDGMENT

Financial support for this research was provided by Project DAAK 60-88-R-0005/N of the U.S. Army Grant, U.S. Army Natick Research, Development and Engineering Center. We acknowledge access to the Varian XL-300 NMR spectrometer made available by the University of Massachusetts High Field NMR Facility. Dr. L. C. Dickinson made the measurements on the IBM 200-AF spectrometer. M.-S.K.-S. was granted a Research Associateship by the Korean Science and Engineering Foundation.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 4th ed.; Association of Official Analytical Chemists: Washington, DC, 1984; Method No. 14.002 and 14.003.

Brevard, C.; Granger, P. *Handbook of High Resolution Multinuclear NMR*; Wiley: New York, 1981; p 50.

Bushuk, W.; Mehrota, V. K. Studies of water binding by differential thermal analysis. II. Dough studies using the melting mode. *Cereal Chem.* **1977a**, *54*, 320.

Bushuk, W.; Mehrota, V. K. Studies of water binding by differential thermal analysis. III. Bread studies using the melting mode. *Cereal Chem.* **1977b**, *54*, 326.

Cesari, L. de. *Sci. Technol. Aliment.* **1974**, *4*, 139.

Chinachoti, P.; Stengle, T. R. Water mobility in starch/sucrose systems: an oxygen-17 NMR study. *J. Food Sci.* **1990**, *55*, 1732.

Clusky, J. E.; Taylor, N. W.; Senti, F. R. Relation of the rigidity of flour, starch, and gluten gels to bread staling. *Cereal Chem.* **1959**, *36*, 236.

Cohen, M. H.; Reif, F. Quadrupole effects in nuclear magnetic resonance studies of solids. In *Solid State Physics*; Seitz, F., Turnbull, D., Eds.; Academic Press: New York, 1957; Vol. 5.

D'Appolonia, B. L.; Morad, M. M. Bread staling. *Cereal Chem.* **1981**, *58*, 186.

Edzes, H. T.; Samulski, E. T. The nature of cross relaxation effects in the protein NMR spin-lattice relaxation of water in biological systems: Hydrated collagen and muscle. *J. Magn. Reson.* **1978**, *31*, 207.

Glasel, J. A. Nuclear magnetic resonance studies on water and ice. In *Water, A Comprehensive Treatise*; Franks, F., Ed.; Plenum Press: New York, 1972; Vol. 1.

Greenspan, L. Humidity fixed points of binary saturated aqueous solutions. *J. Res. Natl. Bur. Stand.* **1977**, *81A*, 89.

Kakalis, L. T.; Baianu, I. C. Oxygen-17 and deuterium nuclear magnetic resonance relaxation studies of lysozyme hydration in solution: field dispersion, concentration, pH/pD, and protein activity dependences. *Arch. Biochem. Biophys.* **1988**, *267*, 829.

Kim, S. K.; D'Appolonia, B. L. Bread staling studies: II. Effect of protein content and storage temperature on the role of starch. *Cereal Chem.* **1977**, *54*, 216.

Krog, N.; Olesen, S. K.; Toernaes, H.; Joensson, T. Retrodegradation of the starch fraction in wheat bread. *Cereal Foods World* **1989**, *34*, 281.

Kulp, K.; Ponte, P. G., Jr. Staling of white pan bread: fundamental causes. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *15*, 1.

Lechert, H. T. Water binding on starch: NMR studies on native and gelatinized starch. In *Water Activity: Influences on Food Quality*; Rockland, R. B., Stewart, G. F., Eds.; Academic Press: New York, 1981.

Leung H. K. Structure and properties of water. *Cereal Foods World* **1981**, *26*, 350.

Leung, H. K.; Magnuson, J. A.; Bruinsma, B. L. Water binding of wheat flour doughs and breads as studied by deuteron relaxation. *J. Food Sci.* **1983**, *48*, 95.

Lindet, L. *Bull. Soc. Chim. Fr.* **1902**, *27*, 634.

Maga, J. A. Bread staling. *CRC Crit. Rev. Food Technol.* **1975**, *8*, 443.

McCune, T. D.; Lang, K. W.; Steinberg, M. P. Water activity determination with the proximitry equilibration cell. *J. Food Sci.* **1981**, *46*, 1978.

Mora-Gutierrez, A.; Baianu, I. C. Hydration studies of maltodextrins by proton, deuterium and oxygen-17 nuclear magnetic resonance. *J. Food Sci.* **1990**, *55*, 462.

Pyler, E. J. *Baking Science and Technology*; Sosland Publishing: Merriam, KS, 1988; Vol. II, pp 592–595.

Rao, P. A. M. S. Thesis, Department of Food Science, University of Massachusetts, 1991.

Richardson, S. J.; Steinberg, M. P. Applications of nuclear magnetic resonance. In *Water Activity: Theory and Applications to Food*; Rockland, L. B., Beuchat, L. R., Eds.; Dekker: New York, 1987.

Schoch, T. J.; French, D. Studies on bread staling. I. The role of starch. *Cereal Chem.* **1947**, *24*, 231.

Shirley, W. M.; Bryant, R. G. Proton-nuclear spin relaxation and molecular dynamics in the lysozyme-water system. *J. Am. Chem. Soc.* **1982**, *104*, 2910.

Slade, L.; Levine, H. Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety. *Crit. Rev. Food Sci. Nutr.* **1991**, in press.

Willhoft, E. M. A. Mechanism and theory of staling of bread and baked goods, and associated changes in textural properties. *J. Texture Stud.* **1973**, *4*, 191.

Wynne-Jones, S.; Blanshard, J. M. V. Hydration studies of wheat starch amylopectin, amylose gels and bread by proton magnetic resonance. *Carbohydr. Polym.* **1986**, *6*, 289.

Zobel, H. F. Review of bread staling. *Baker's Dig.* **1973**, *47*, 52.

Received for review December 4, 1990. Revised manuscript received June 19, 1991. Accepted July 11, 1991.

Registry No. SE 1170, 136327-55-4; H_2O, 7732-18-5; glycerol, 56-81-5; sorbitol, 50-70-4; sodium stearoyl-2-lactylate, 25383-99-7.