Carbohydrate Polymers **6** (1986) 289–306

Hydration Studies of Wheat Starch, Amylopectin, Amylose Gels and Bread by Proton Magnetic Resonance

S. Wynne-Jones* and J. M. V. Blanshard

Department of Applied Biochemistry and Food Science, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leicestershire LE12 5RD, UK

(Received: 30 October 1986)

SUMMARY

Changes in the state of water, with time, in gels and bread have been investigated using pulsed proton magnetic resonance to measure spin–spin relaxation times and the amount of unfreezable water in the system.

Relaxation studies of wheat starch gels with water contents in the range 25–60% show a reduction in the T_2, within the first 48 h after formation, indicating an overall decrease in mobility of the water molecules. Freezing experiments show that this decrease in the relaxation time is accompanied by an increase in the fraction of 'bound' water in the gel. The rate and extent of this change exhibits a positive temperature dependence, whereas general retrogradation phenomena (such as increase in crystallinity and firming) show a negative temperature coefficient. The activation energy calculated for this process is consistent with hydrogen bond formation.

Examination of the two major fractions of starch (amylopectin and amylose) shows that the amylopectin gels closely follow the behaviour of starch gels, whereas amylose gels show no change in the state of the water on storage.

The accessibility of the bound water in the retrograded gels has been tested by mixing with deuterium oxide. These experiments show that all the water in the gel is available for exchange and can be replaced with heavy water.

* Present address: 136 Bramcote Lane, Chilwell, Nottingham, UK.

Carbohydrate Polymers 0144-8617/86/$03.50 —

Samples of heated gluten and water show a greater 'binding' of water in the protein than in starch and that once bound the state of the water does not change.

Studies on bread, stored in sealed glass tubes, show no significant change in the relaxation times or levels of bound water over a period of 7 days, even though staling has occurred and the bread has apparently dried out. The bread examined was stored, without crusts, in sealed moisture-proof bags in an oxygen free atmosphere in order to minimise the effects of water migration and microbial attack. Any changes in the relaxation times and fractions of bound water observed in these samples can be accounted for by loss of 'free' water only. These results have also been confirmed using differential scanning calorimetry.

Reheating of stale bread, which is known to refreshen it, produces no significant change in the relaxation behaviour of the water, even though a decrease in the 'B' crystallinity is observed.

INTRODUCTION

The physical state of water in food systems is believed to play an important role in its properties. The concept of 'bound' and 'free' water has been used to describe the states of water, but the physical meaning of these descriptions is controversial as the levels measured depend on the technique used. Bound water in this case is taken to be that quantity of water molecules whose molecular motions have been modified from that of pure water. Nuclear magnetic resonance is a very sensitive probe of molecular motions in the fluid state.

The model of the state of water used here is one where a fraction of the total water has its molecular mobility reduced due to hydrogen bonding to a macromolecular surface, and the rest is essentially free, liquid water. The characteristic spin–spin relaxation times of these two states are an order of 1000 times different, with free water having a T_2 of approximately 2 s and bound water having an intrinsic T_2 of about 1 ms. If the two types of water are physically distinct then one would obtain a decay profile consisting of two exponential processes with time constants of those above and relative amplitudes determined by the quantity of water in each of the states.

However, in the time scale of the experiment water molecules can diffuse between the two states; thus the relaxation time of water molecules in this type of system is an average between that of free and

bound water. It has been shown (Zimmerman & Brittin, 1957) that the relaxation time of a system undergoing rapid exchange is given by:

$$\frac{1}{T_2} = \frac{P_b}{T_{2b}} + \frac{P_f}{T_{2f}}$$

where T_2 is the measured relaxation time and P_b, P_f, T_{2b} and T_{2f} are the fractions and relaxation times of the bound and free water populations, respectively. Thus any change in the relative quantity, or state, of 'binding' will produce an overall change in the relaxation time.

Ice exhibits a very fast spin–spin relaxation time, of the order of microseconds, and thus due to the dead time of most NMR spectrometers any signals from ice are not detected. This facilitates the determination of the quantity of unfrozen water, which is assumed to be bound. The intensity of the free induction decay signal from any sample is proportional to the number of mobile protons, which at temperatures below 0°C gives a direct measure of the fraction of unfrozen water.

Differential scanning calorimetry (DSC) has also been used in determining levels of bound water in dough and bread (Bushuk & Mehrotra, 1977) and despite quantitative differences being observed between the two techniques they agree qualitatively.

MATERIALS AND METHODS

Preparation of wheat starch, amylopectin, amylose and gluten gels

Wheat starch was kindly supplied by ABR Chemicals Ltd (Corby, UK) and a moisture determination showed it to contain approximately 11% water by weight. Amylopectin (from waxy maize) and amylose (from potato) were supplied by Sigma Chemical Company, USA, while wheat gluten was obtained from BDH Chemicals Ltd (Poole, UK).

Two methods of gel preparation were employed, namely microwave heating and extrusion. Samples prepared by microwave heating were mixed with water, to give a total weight of approximately 50 g, and placed in a petri dish which was then sealed with tape to prevent sample loss. The petri dish was then placed in the microwave oven, on a rotating turntable, and heated for approximately 2 min at a power

rating of 500 W. Every 30 s the sample was inspected and inverted on the turntable in order to achieve a homogeneous heating regime.

For extrusion a Brabender single screw extruder (Model 200N) was used under mild extrusion conditions, with a 1:1 compression screw and a heater temperature profile of 105°C, 100°C and 50°C from the feed hopper to the die piece. The relatively low die piece temperature was used in order to minimise 'flash off' of water from the sample on leaving the extruder. At a screw revolution rate of 150 rpm the torque produced was in the range 2–10 Nm for water contents in the range 60–25% (w/w)*. Microscopic examination of the resultant gels showed no damage to the swollen starch granules.

After 15 min, to allow the gels to reach room temperature, samples were transferred to 8 mm NMR tubes and sealed, and the tubes stored at a number of temperatures. Samples were also sealed in polythene bags under the same temperature conditions.

Bread baking

Commercial hard wheat flour was used in the preparation of bread loaves by the Chorleywood process. The recipe used was: 1·5 kg hard wheat flour, 0·9 kg water, 30 g salt, 40 g dried yeast, 10 g sugar, 45 mg potassium bromate and 45 mg ascorbic acid.

The ingredients were mixed first by hand, then transferred to a Z-blade mixer and mixed for 3 min at high speed. The resultant dough was left to rise for 1 h, separated into five loaves, placed in bread trays and allowed to prove for a further 20 min. The loaves were then baked at 235°C for 20 min and after cooling for 30 min had their crusts removed and the crumb was stored in sealed, evacuated, polythene bags at a number of storage temperatures. Samples were also transferred to NMR tubes, sealed and stored at the same temperatures.

Moisture content analysis

The moisture contents of the prepared samples were determined by drying samples in a vacuum oven for 48 h at 60°C.

*Water contents are represented as %(w/w), e.g. 25% (w/w) signifies 25 g water per total 100 g system (75 g starch + 25 g water). This also is the water content on a wet weight basis. On a dry weight basis, the same system has a water content of 25/75 = 33·3%.

NMR

Proton spin–spin relaxation times were obtained at room temperature, on a 30 MHz pulsed NMR spectrometer, using the Carr–Purcell–Meiboom–Gill pulse sequence. The decays, consisting of 256 echos, were captured and averaged on a DataLab 4000 signal averager, and the resultant decay data were stored on floppy disc on an Apple II + microcomputer. The relaxation times were determined by the Newton–Raphson non-linear least squares method using a program developed by the authors.

Freezing studies were performed by monitoring the signal intensity of the free induction decay (FID) as the sample temperature was reduced from 20°C to −30°C. A period of 15 min was allowed between temperature jumps for the sample temperature to equilibrate. Changes in the sensitivity of the spectrometer with temperature were monitored by performing the same experiment on a sample of ethanol; by this method the amplitudes of the FIDs could be normalised. To eliminate any freeze–thaw damage affecting the results, new samples were taken for each bound water determination.

X-ray and DSC studies

X-ray diffraction patterns were obtained on a Philips APD-15 powder diffractometer using Cu K-α radiation. Freezable water determinations were carried out on a Perkin-Elmer DSC II-B using the melting mode as described by Bushuk & Mehrotra (1977).

RESULTS

Wheat starch gels

Figure 1 shows the time evolution of the spin–spin relaxation time for a 50% water (w/w) starch gel prepared by the microwave technique. The curve shows two processes, a fast one over the first 4 h giving an increase in the T_2 and a slower one which proceeds over the next 24 h leading to a reduction in the relaxation time. This initial rise indicates an increase in the mobility of the water, with the second process showing a decrease. The initial increase in mobility can be accounted for by redistribution of the water in the sample.

Examination of the gels prepared by the microwave technique shows that the samples are still heterogeneous with regions of clear gel

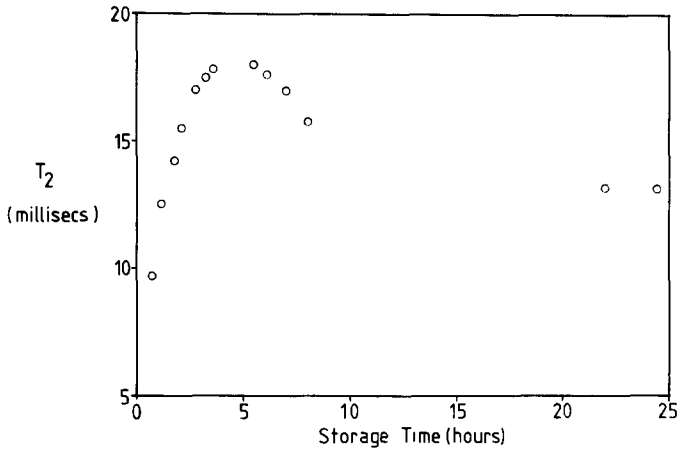

Fig. 1. Time evolution of the NMR spin–spin relaxation time (T_2) for a 50% water/starch (w/w) gel prepared by the microwave technique.

and white powder. Water moving from the powder state to the gel regions will show the observed increase in mobility. The slower process of reduction of T_2 is similar to that observed previously in this laboratory (Capelin & Blanshard, unpublished results) and shows a decrease in the overall mobility of the water molecules. Due to the sensitivity of the results to the preparative technique of microwave heating, this method is not advised.

Gels prepared by the extrusion method show no signs of inhomogeneity, and have produced clear, uniform gels with water contents approximately 5% (w/w) less than that of the feed mixture. Figure 2 shows the change in T_2 on storage for a 45% (w/w) water/starch gel, prepared by extrusion and stored at a number of temperatures between -5 and 37°C. The first point to note is that there is no apparent fast process confirming that there is a higher degree of homogeneity in these samples compared with the gels prepared by microwaving. As can be seen the process exhibits a positive temperature dependence, which is in contrast to the accepted negative temperature coefficient for retrogradation obtained from X-ray and DSC work. Calculation of the activation energy (Fig. 3) of this process gives a value of approximately 15 kJ mol^{-1} which is consistent with the formation of hydrogen bonds which, with the decrease in the relaxation time, points to an increase in the binding of water.

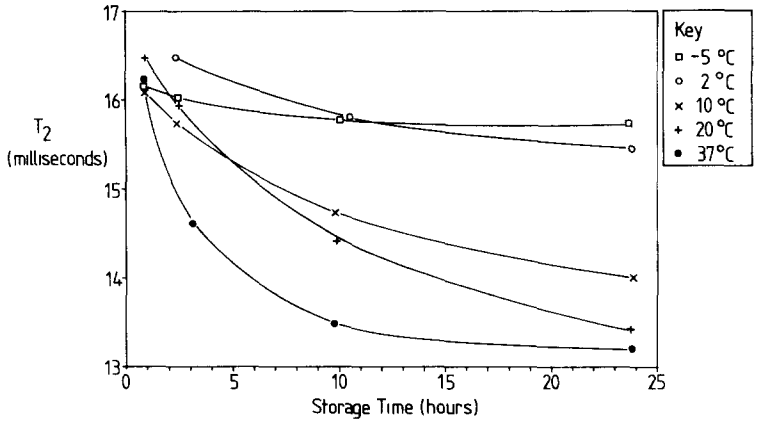

Fig. 2. Time evolution of the NMR spin–spin relaxation time (T_2) for a 45% water/starch (w/w) gel prepared by extrusion and stored at temperatures −5, 2, 10, 20 and 37°C.

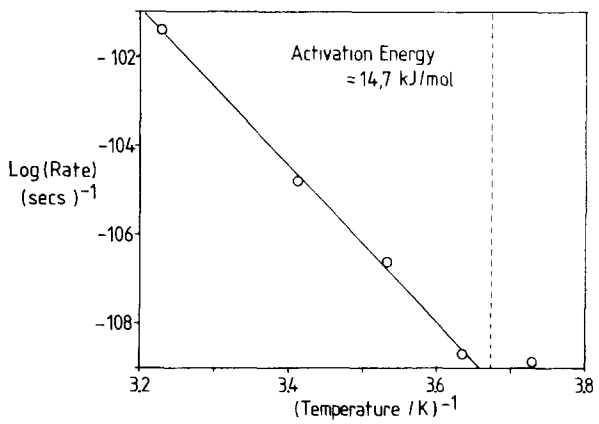

Fig. 3. Plot to determine activation energy of changes in T_2 of a 45% water/starch (w/w) gel system.

This decrease in relaxation time has been observed for a range of water contents between 25% and 60% (w/w). The rate and extent of the change is maximum at about the 45% (w/w) water level. At water contents lower than this it is assumed that the molecular mobility of water is hindered by the increased rigidity of the gel and the time-dependent fractional increase in the bound water asymptotically

approaches zero when the total water level of samples is reduced to approximately 30% (w/w), at which point all the water is bound. At high water contents the fraction of bound water is smaller and the expected changes in this small amount do not greatly affect the relaxation behaviour. The increase in bound water can be seen in Fig. 4, which was performed on a 35% water/starch (w/w) gel, which shows a change in the level of bound water (as a percentage of the total water) over 48 h from 49% to 64%. Changes were observed after 48 h but it was apparent that loss of water from the sample to the storage bag had taken place resulting in a falsely high reading of the bound water fraction.

The overall effect of water changes in starch gels is that of an increase in bound water over 48 h leading to a reduction in the overall mobility of the water molecules. The process appears to be concurrent with retrogradation but is not a consequence of it.

In order to determine which component of the starch was responsible for the binding, gels of amylopectin and amylose were examined. It was evident from their behaviour on mixing with water that the two components exhibit different hydration properties. Amylopectin was very similar to starch when mixed with water and produced a moist powder up to 40% water (w/w), a 'dough' at water contents in the

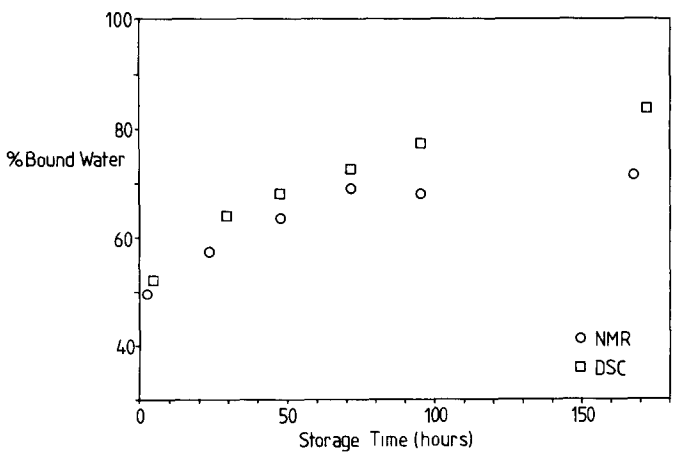

Fig. 4. Change in the bound water (as a percentage of the total water) in a 35% water/starch (w/w) gel.

range 45–55% (w/w) and a slurry at water contents above 55% (w/w). On extrusion, these formed uniform, clear gels. Amylose, however, showed a greater affinity for water, remaining as a powder up to 60% (w/w) water and producing a slurry at higher water contents. Extrusion of amylose with less than 50% (w/w) water resulted in no apparent change, whilst higher water contents produced a uniform opaque 'gel'.

Figure 5 shows the change in T_2 on storage for a 30% water/amylopectin (w/w) gel. The same reduction in relaxation time is observed as for the starch gels, with a slight indication of the fast process exhibited by the gels prepared by the microwave method. The rate of decrease in T_2 is faster than in a comparable starch gel, and this faster rate of decrease was also observed in studies of retrogradation of amylopectin gels using other physical techniques such as X-ray diffraction and firmness measurements.

The time dependencies of relaxation times for a 50% and 60% (w/w) water/amylose 'gel' are shown in Fig. 6. As can be seen, no significant changes occurred, and no signs of retrogradation were apparent either.

Hence the change of state of water in starch gel systems is due solely to the amylopectin fraction, with the rate of change possibly moderated by the amylose.

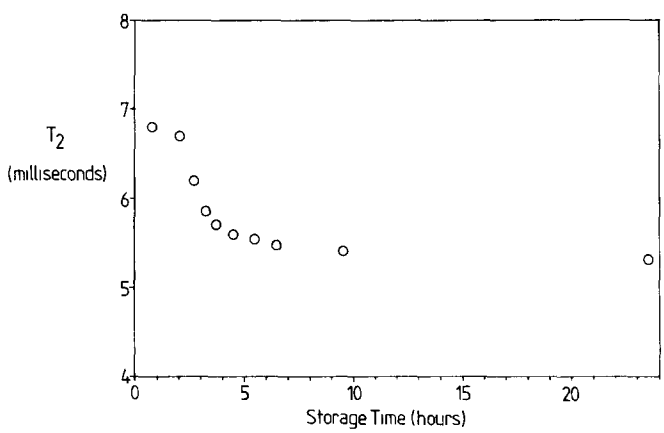

Fig. 5. Time evolution of the NMR spin–spin relaxation time (T_2) for a 30% water/amylopectin (w/w) gel.

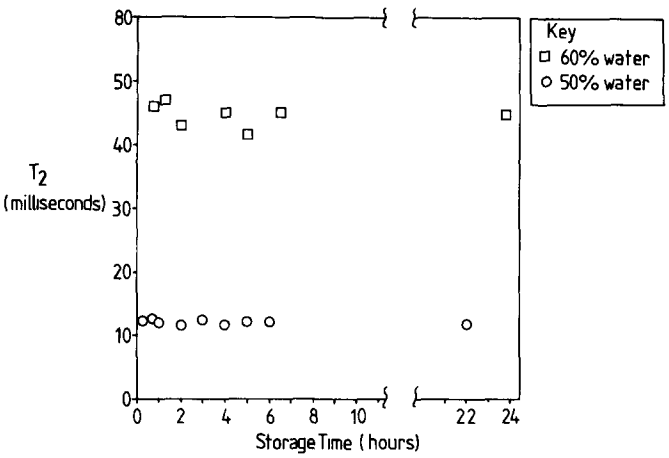

Fig. 6. Time dependence of the NMR (T_2) relaxation time for a 50% and 60% water/amylose (w/w) gel.

One proposed mechanism for the reduced mobility of the water molecules was that of the formation of pockets of trapped water during the retrogradation process. To test this hypothesis, samples of retrograded gels were mixed with heavy water to determine whether the trapped water was unable to exchange with the heavy water. If this were so, one would only see a loss of the free water component in the NMR signal. The results of these exchanges showed that all the water in the gel could be rapidly replaced (in approximately 2 h) by heavy water, and no evidence of trapped water could be seen.

Before examination of bread could proceed, samples of heated gluten were studied in order to characterise changes in hydration on storage. The results were very similar to those of the amylose gels, with the gluten/water system showing no change in T_2 over a period of 2 weeks.

Bread studies

The initial work on starch gels had shown that the T_2 was a sensitive indicator of the state of the water in the systems. Table 1 shows the relaxation time for a standard bread crumb sample stored in sealed NMR tubes. Under these conditions no change in the state of the

TABLE 1
Variation of Relaxation Time for Standard Chorleywood Bread at Two Storage Temperatures

Storage time (h)	*Relaxation time (ms)*
Storage temperature 2°C	
1·6	8·69
2·0	8·85
3·0	8·25
4·0	8·17
6·0	8·90
23·5	8·83
98·0	8·86
Storage temperature 37°C	
1·5	8·69
1·9	8·67
2·9	8·22
3·9	8·24
5·9	9·00
23·4	8·50
97·9	8·50

water is evident, which is surprising in view of the previous results which show that, in starch gels, the T_2 does change. This impasse can be overcome if we assume that there is an uneven distribution of water between the gluten and the starch components. If the gluten absorbs per gram more water than the starch, leaving the starch hydrated to a level of approximately 30% water (w/w), then the relative increase in bound water of the starch system is minimal, as shown before, resulting in no apparent change in relaxation behaviour as a consequence of the starch component. The gluten, as observed before, does not change, thus resulting in no change in relaxation behaviour. Redistribution of water between the fractions on storage may also lead to this result, a view which will be discussed later.

Examination of the fractions of bound water in the bread, stored in sealed plastic pouches, showed an increase (Fig. 7). This, at first puzzling, observation can be explained by the loss of free water from the bread on storage in pouches, which was not possible in the sealed NMR tubes. Figure 7 also shows the change in relaxation time for

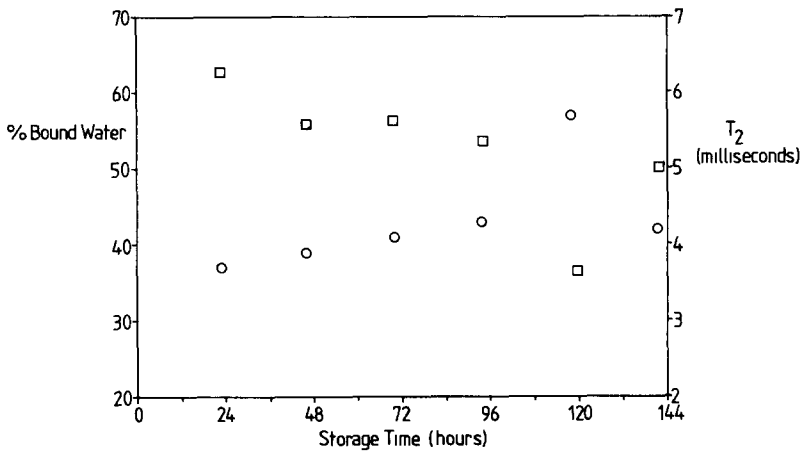

Fig. 7. Changes in NMR (T_2) relaxation time (□) and bound water (as a percentage of total water) (○) for bread samples stored in sealed plastic pouches.

these same bread samples taken from the plastic pouches. The decrease in the relaxation time observed is matched by an increase in the fraction of bound water, and is seen most dramatically in the sample after 115 h. The variation in the results after 4 days was seen to be large, but could be shown to be entirely due to loss of free water from the bread.

Further evidence of the change in the free water fraction can be seen by DSC. Samples of bread were heated from −40°C to 100°C and the enthalpy of the ice melting endotherm determined. From the heat of fusion of water the weight of ice could be calculated. The total water content of each sample pan was determined as were the fractions and absolute quantities of bound and free water. The determination of bound water by this method gave consistent results with the NMR, with a systematic error of 5% at most between the methods.

Figure 8 shows the change in the amounts of bound and free water on storage for bread given as a percentage of the sample weight. This figure shows a small change in the quantities of bound and free water up to 4 days (as seen by NMR). After this period, loss of free (freezable) water is seen but with no significant change in the quantity of bound water. Thus the change of water in bread on storage is minimal during the first 4 days, but thereafter loss of free water is shown to be

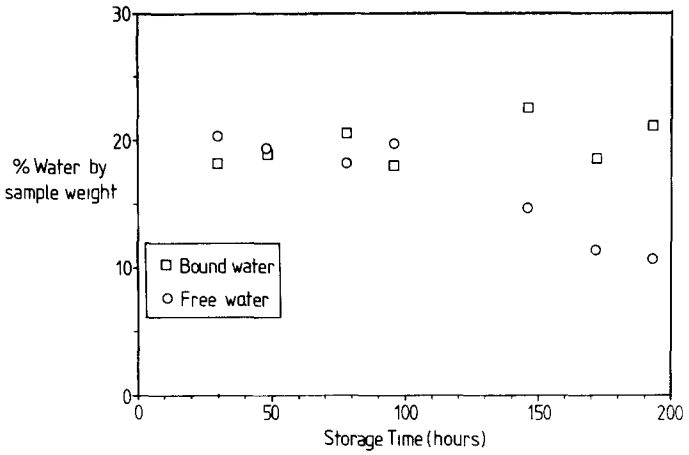

Fig. 8. Changes in the amounts of bound and free water on storage of bread recorded as a percentage of sample weight.

important. 'Drying out' of bread on staling is said to occur after 2 days of storage, but the organoleptic sensation of dryness is complicated by the firming process. The effect of moisture migration from the crumb to the crust also plays a major part in this process.

Bread refreshening studies

Pisesookbunterng *et al.* (1983) have shown that heating 'refreshens' bread. If this treatment is carried out within the first 2 days of storage, bread with similar qualities to freshly baked bread is obtained, but heat treatment after 2 days gives a firmer crumb than that of fresh bread. They attribute the change in the first 2 days to the heat reversible retrogradation of starch whereas, after that, other processes govern the extent of staling in bread.

Bread stored for 7 days at 2°C, which had staled in terms of firmness and retrogradation of the starch fraction, was heated in sealed pouches to a variety of temperatures between 20°C and 60°C, for 45 min, in order to study the effect of refreshening on the water relaxation.

X-ray analysis showed that although the crystallinity decreased to approximately 15% of that in the retrograded bread after heating at

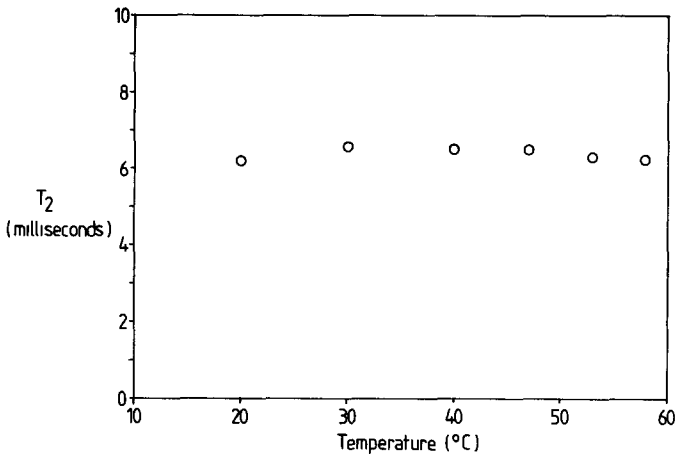

Fig. 9. NMR (T_2) relaxation times of bread samples (previously stored for 7 days at 2°C) reheated to temperatures between 20 and 60°C. NMR measurements were taken at ambient temperature.

58°C, and the bread was less firm, the relaxation measurements showed no change in the state of the water (Fig. 9). This suggests that the decrease in firmness observed on heating is not due to a change in the state of the water.

DISCUSSION

The results of the change in the state of water on storage for the starch gel systems can be summarised as follows:

(1) The water mobility decreases over the first 48 h of storage and exhibits a positive temperature dependence. The positive temperature coefficient is in conflict with the negative temperature regime of the retrogradation process, as observed by calorimetric (Colwell *et al.*, 1969) and X-ray analysis (Wright, 1971). Determination of the activation energy shows that hydrogen bonding could be the rate-determining process in the increase of the bound water.

(2) Determination of the rate of the water binding process and that of the retrogradation process shows that the water binding process is faster, being complete after approximately 48 h, whereas the retro-

gradation process may take up to a week to be complete. Thus it is apparent that the change in state of the water cannot be correlated to the retrogradation process directly.

(3) For wheat starch gels it was found that the level of binding corresponds to a water content of approximately 30% (w/w). This compares favourably with the figure of 25–27% found in dough systems by Bushuk & Mehrotra (1977) and by Lechert *et al.* (1980) in studies of starch hydration. As the change in the NMR response is due to relative changes in the level of this bound state it is apparent that the technique is most sensitive at about 40–45% water level. At concentrations near to 30%, practically all the water is bound and any increase represents only a small change in a large level of bound water, whereas at much higher water contents the fractional change in the state of the water is too small to be measured.

The bound water in this system is not 'trapped' and is available to exchange by heavy water; this has also been shown in native starches by Hennig & Lechert (1977). It has further been shown that the hydroxyl hydrogens on the starch components are completely exchangeable in heavy water (Taylor *et al.*, 1961).

Changes in the state of water in bread showed (Fig. 7) that the level of bound water increased, but only by 2–3%, during the first few days, showing reduced though similar properties to those observed in the starch gel system. The fact that no significant changes in the *relaxation times* were observed can be accounted for by noting that the level of water in the system is close to that of the bound water level and the changes of 2–3% noted above would not result in any great shift in the relaxation behaviour. Leung *et al.* (1983), however, showed a greater decrease in relaxation times for bread using heavy water. The decrease of relaxation time observed occurred over the first 2 days, as seen here.

Two factors must be considered in order for a full comparison to be made. First, Leung *et al.* (1983) stored their bread with crust, and loss of water from the crumb to the crust accounted for approximately 40% of the reduction in relaxation times. Secondly, the use of deuterium oxide has enhanced the effects, compared with water, as they have shown that heavy water associates more strongly with flour, due to the increased strength of the 'hydrogen bonds' formed. Thus any increase in the level of bound water will produce a greater change in the relaxation behaviour.

If we take these factors into account we can see that the results are essentially similar.

If we now focus attention more especially on the unfreezable water, one possible mechanism that has been proposed for the increase in bound water on staling is that the water is incorporated into the 'B' crystals which form in the starch component on retrogradation. Each unit cell of the crystal has 36 water molecules which corresponds to 25% water, by weight. In a fully retrograded system the level of crystallinity is of the order of 15% of the total starch. With starch being approximately 70% by weight of the dry solids in bread, this means that the loss of water to the crystals is of the order of 1–2%, a smaller amount than observed. The retrogradation process is also known to occur over the time scale of a week whereas the majority of the increase in binding is seen over the first 2 days.

Refreshening of bread, with a consequent loss of crystallinity, produced no change in the relaxation behaviour observed here. All of these factors lead to the conclusion that the change in the state of water in bread is not governed by the retrogradation process, but by some other process in the starch component, which leads to increased hydrogen bonding with the water.

A somewhat fuller understanding of the problem may be gained by following Willhoft (1971), who has studied the distribution of water between gluten and starch in doughs and breads by the water balance method. He finds that the water in a dough is distributed unequally between the two components, such that in a dough containing 44% water, by total weight, with a gluten:starch ratio of 1:6 the water contents of the two phases are 126% and 73% by dry weight of the gluten and starch, respectively. After baking, approximately 3% of the total water is lost and the water contents are 105% and 71%, by dry weight, for the gluten and starch. These figures correspond to water contents by wet weight of 49% (w/w) and 41·5% (w/w), respectively. On standing at 20°C the water then redistributes itself between the two phases such that water is transferred from the gluten to the starch. This process is complete after 24–48 h. The final distribution of water measured corresponds to a water content of 47·4% (w/w), in the gluten, and 42·5 (w/w), in the starch phase, by wet weight.

From examination of the rate of redistribution, Willhoft concluded that the rate-determining step was the diffusion of water from the gluten to the starch. However, in real doughs the gluten forms a

thin membrane around the starch granules and he believed this would present no barrier to diffusion from the gluten. He proposed that the limiting process in real doughs and breads is due to either the rate of release of the bound water from the gluten or the uptake of this water, via diffusion, by the starch.

If we consider a simple model where any water transferred between the two phases passes via the free water state from the gluten to the starch and remains in the free state, then the effect of this added water on the starch is to yield a decrease in the overall fraction of bound water by 3–4%. Such a model does *not* fit the experimental results.

The preferred model, presented here, which represents the change in the state of water on aging of bread is one where the water binding of the starch phase increases with time, as in the simple starch gel systems, but concurrently with this water is also transferred from the gluten phase and enters the starch phase, as free water. The quantities of water and the rates involved in these processes are comparable and thus the overall effect is to produce a minimal change in the fraction of bound water and, consequently, little change in the relaxation behaviour of the system. This change, as shown by Willhoft (1971), is non-heat reversible. Thus refreshening of the bread produces no change in the system, as seen by nuclear magnetic resonance.

ACKNOWLEDGEMENTS

The authors warmly acknowledge the preliminary studies of Dr S. Capelin and the support given to that work by the Agricultural and Food Research Council.

REFERENCES

Bushuk, W. & Mehrotra, V. K. (1977). *Cereal Chem.* **54**, 320–5.

Colwell, K. H., Axford, D. W. E., Chamberlain, N. & Elton, G. A. (1969). *J. Sci. Fd. Agric.* **20**, 550–5.

Hennig, H. J. & Lechert, H. (1977). *J. Colloid and Interface Science* **62**, 199–204.

Lechert, H., Maiwald, W., Kothe, R. & Basler, W.-D. (1980). *J. Food Processing and Preservation* **3**, 275–99.

Leung, H. K., Magnuson, J. A. & Bruinsma, B. L. (1983). *J. Food Science* **48**, 95–9.
Pisesookbunterng, W., D'Appolonia, B. L. & Kulp, K. (1983). *Cereal Chem.* **60**, 301–5.
Taylor, N. W., Zobel, H. F., White, M. & Senti, F. R. (1961). *J. Phys. Chem.* **65**, 1816–20.
Willhoft, E. M. A. (1971). *J. Sci. Fd. Agric.* **22**, 176.
Wright, W. B. (1971). Starch crystallinity and bread staling, Paper presented at *Symposium on Starch Granule Structure and Technology*, London, Soc. Chem. Ind., Food Group, London.
Zimmerman, J. R. & Brittin, W. E. (1957). *J. Phys. Chem.* **61**, 1328.