

# Effects of Temperature on Sorption of Water by Wheat Gluten Determined Using Deuterium Nuclear Magnetic Resonance

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## ABSTRACT

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The effects of lipids and residual starch components of wheat flour gluten on gluten hydration properties were investigated using nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) techniques. Whole or native, lipid-free, starch-free, and lipid- and starch-free gluten samples were prepared from wheat (*Triticum aestivum*) cv. Mercia. <sup>2</sup>H NMR relaxation on gluten samples hydrated with deuterium oxide (D<sub>2</sub>O) was measured over a 278–363 K temperature range. FTIR spectra were recorded in dry and fully hydrated material. Transverse relaxation (T<sub>2</sub>) results indicated that all four gluten samples were hydrophilic in nature. There was little difference in relaxation behavior of whole and lipid-free gluten samples. T<sub>2</sub> values and populations of the relaxation components were very similar in each. The FTIR spectra of both samples showed an increase in extended

β-sheet secondary structures on hydration. These results suggest that lipid binding in gluten, if it occurs, has little effect on wheat gluten properties. Adding starch to the gluten matrix results in an increase in water sorption on heating that may be attributed to the effects of starch gelation. However, the whole water uptake of the gluten cannot be accounted for by the contribution of the residual starch, as estimated by the effects of added starch. Extraction of residual starch required solubilization of the protein, including breaking of the disulfide bonds. This process altered the gluten structure and properties. Light microscope investigation showed that gluten with residual starch extracted were unable to form fibrillar strands on hydration. NMR and FTIR results showed greater water sorption in both samples with extracted starch than in the unextracted samples.

Much about the chemical nature of wheat gluten is already known. Extracted, dry gluten contains ≈80% protein, the vast majority of which are prolamins (Tatham et al 1990). The remaining 20% of gluten is composed of roughly equal amounts of starch and lipid. The prolamins can be divided into either monomeric or aggregative proteins and have similar amino acid compositions, each containing relatively high amounts of both glutamine and proline. Mixing gluten with water causes an initial breakdown of the larger protein aggregates, followed by the build-up of a continuous and complex polymer structure (the gluten matrix) (Weegels et al 1996). It is generally accepted that the properties of the matrix largely determine wheat dough elasticity and extensibility, both essential rheological properties in the breadmaking process, and that variation in such properties may be governed by two compositional parameters, ratio of polymeric-monomeric proteins and molecular size distribution of the polymeric protein (MacRitchie 1992). However, the exact nature of the gluten matrix is unknown, and the part played by non-protein constituents of gluten in determining structure and properties is unclear.

The nonstarch lipid content of wheat flour, which constitutes 80% of the total lipid content, influences baking qualities and is technologically very important (Morrison 1988). Up to 20% of flour lipids are found alongside the amylose within the wheat starch granule (starch lipid), but it is unlikely they influence other components. Nonstarch lipids are either nonpolar or polar, and are commonly divided into free lipid (extractable by nonpolar solvents) and bound lipid (not extractable under these conditions). The nonstarch, free lipid of wheat flour represents ≈90% of the non-polar lipid and 20% of the polar lipid, while the bound lipid of the flour represents the remainder. Increased levels of polar lipid, especially glycolipid, may be responsible for the improved baking performance of wheat flours, while nonpolar lipid may have a detrimental effect (De Stefanis and Ponte 1976).

A large proportion (≈45%) of wheat flour lipid is lost during the washing stage in gluten preparation, but the remainder is difficult to extract (Zawistowska et al 1985a). Gluten washing also removes considerably more nonpolar than polar lipid. However, the remaining lipids may be approximately equally divided between the gliadin and glutenin fractions. There is much evidence for the involvement of the gluten proteins in lipid binding during dough development (Carr et al 1992). Specific proteins with high affinity to flour lipids have been identified (Zawistowska et al 1985a). Results from spectroscopic measurements have indicated strong lipid-protein interactions (Wehrli and Pomeranz 1970, Nishiyama et al 1981, Genot et al 1984) and an amphiphilic character of the polar lipids has been proposed (Chung 1986). It is suggested that such characteristics enable these molecules to strengthen the gluten by effectively bridging protein and starch components. However, recent work suggests that observed lipid binding may involve lipid mesophase transformation and the nonspecific occlusion of lipid phases within the gluten network (Carr et al 1992).

Starch is by far the most abundant component of wheat flour, but its part in determining the breadmaking performance of flour is unclear. Starch-protein interactions are known to occur (Zawistowska et al 1985b) and are especially important in determining wheat endosperm hardness (Morrison et al 1992). Evidence suggests that interactions of starch granules with the high molecular weight (HMW) proteins of wheat occur to a greater extent than with the low molecular weight (LMW) proteins (Eliasson and Tjerneld 1990). However, the main role of starch may be in determining the water absorption capacity of a dough and crumb firming of the baked product (MacRitchie 1984). Gluten washing during the preparation stage successfully removes the majority of the starch but a small proportion of residual starch remains.

One way to test the influence of the various matrix components is to examine their effects on the water sorption capacity of the matrix. Lipids and starch may have opposite effects because the former are hydrophobic while the latter are hydrophilic and can adsorb large amounts of water on gelation. In this study, we have examined the effect of the lipid and starch content on the hydration properties of wheat flour gluten (whole gluten, lipid-free gluten, starch-free gluten, lipid- and starch-free gluten) using nuclear magnetic resonance (NMR) relaxation and Fourier transform infrared (FTIR) measurements. The approach used in the NMR work was to examine the effects of temperature on water absorption using D<sub>2</sub>O and observe the ingress of water into the protein matrix as

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temperature was increased. FTIR measurements allowed an assessment of the changes in secondary structure resulting from hydration and sample treatment. The effects of hydration and mixing on the microscopic structure of the samples were also examined using light microscopy.

## MATERIALS AND METHODS

### Materials

Four samples of gluten (Table I) were prepared from commercial white flour of wheat (*Triticum aestivum*) cv. Mercia. Whole gluten (sample 1) was prepared by mixing the flour with water to a thick dough and handwashing under a stream of distilled water which removed the water-soluble components and most of the starch. The resulting gluten was rapidly frozen in liquid nitrogen and freeze-dried. Protein content of sample 1 measured using a Kjeldhal method was  $\approx 80\%$ . Lipid content was calculated as the difference between the protein content of lipid-free gluten and that of sample 1. Residual starch was estimated as 100% minus the protein content of lipid-free gluten.

Lipid-free gluten (sample 2) was prepared from defatted flour. Flour was mechanically stirred at room temperature in dry butanol-1-ol using a solvent-to-flour ratio of 10:1. The mixture was filtered and the procedure was repeated with chloroform. The resulting flour was air-dried and washed as above to produce gluten. Protein content was  $\approx 85\%$ .

Starch-free gluten (sample 3) was prepared from sample 1 whereas lipid- and starch-free gluten (sample 4) was prepared from sample 2. Powdered freeze-dried gluten was mechanically stirred for 1 hr at room temperature in an aqueous solution of 50% (v/v) propan-1-ol, 2% (v/v) 2-mercaptoethanol and 1% (v/v) acetic acid. This procedure was repeated twice and the supernatants, after centrifugation, were pooled and dialyzed against distilled water for 60 hr at 4°C. The resulting material was then freeze-dried. All gluten samples (Table I) were stored in a desiccator at +1°C until required.

To further determine the effects of starch on gluten relaxation properties, two gluten samples with increased levels of starch were prepared (Table II) using methods similar to Eliasson (1983). Sample 1a was prepared by mixing 25 mg of sample 1 with 2.5 mg of wheat starch (Fisons Laboratory Reagents, Loughborough, Leicestershire, UK) previously dried in a vacuum oven at 313 K for 24 hr. Sample 1b was prepared as sample 1a, except 5 mg of starch was added. Samples were placed in NMR tubes and mixed by hand.

### Light Microscopy

Flour particles are known to spread on an air-water interface (Amend and Belitz 1991). To assess the spreading behavior of the four gluten samples, particles of each sample were sprinkled onto

a drop of distilled water on a slide and allowed to spread for 1 min before a glass coverslip was applied. The samples were examined and photographed unstained.

### NMR Experiments

Samples 1–4 (25 mg), 1a (27.5 mg), and 1b (30 mg), were hydrated in 150 mg of D<sub>2</sub>O in 5-mm NMR tubes. After stirring the samples by hand, tubes were sealed and left at ambient room temperature for 24 hr. <sup>2</sup>H transverse relaxation measurements (Fourier transform NMR) were then made using the Carr-Purcell-Meiboom-Gill (CPMG) sequence over a maximum temperature range of 278–363 K at 46.05 MHz on a Bruker MSL 300 spectrometer using a probe fitted with a 10-mm bore saddle coil. Pulse lengths of 90 and 180° were 15 and 30  $\mu$ sec, respectively. Pulse spacing was 4 msec. The relaxation data (component time constants T<sub>2</sub> and % population) were analyzed using version 3.10 of Tablecurve curve fitting software (Jandel Scientific, San Rafael, CA).

NMR relaxation and its measurement are described by Colquhoun and Goodfellow (1994). The spin of charged nuclei generate a magnetic moment which, in an external magnetic field, will experience a torque, tending to align it with the field in a state of equilibrium (i.e., in the direction of the z axis). A burst of radio frequency radiation is then applied to excite all the frequencies of interest, in this case that of deuterium, and magnetization shifted in the direction of the x axis. Decrease of this magnetization, which is due to the relaxation of the spins from the excited to the equilibrium state, gives data from which time constants T<sub>2</sub> (the inverse of relaxation rates) and component populations can be monitored with time and calculated. In the CPMG sequences used, T<sub>2</sub> relaxation curves are exponential and may comprise many components (multiexponential). However, often the relaxation can be adequately described by two

TABLE I  
Gluten Samples

Sample	Description	Main Constituents (% w/w)		
		Protein	RS <sup>a</sup>	Lipid
1	Whole or native	80	15	5
2	Lipid-free	85	15	0
3	Starch-free	95	0	5
4	Lipid and starch-free	100	0	0

<sup>a</sup> Residual starch.

TABLE II  
Whole Gluten Samples with Increased Starch Levels<sup>a</sup>

Sample	Weight (mg)		Main Constituents (% w/w)				Lipid
	Gluten	AS	Protein	RS	AS	TS	
1a	25.0	2.5	72.7	13.7	9.1	22.8	4.5
1b	25.0	5.0	66.7	12.5	16.7	29.2	4.1

<sup>a</sup> RS = residual starch; AS = added starch; TS = total starch.

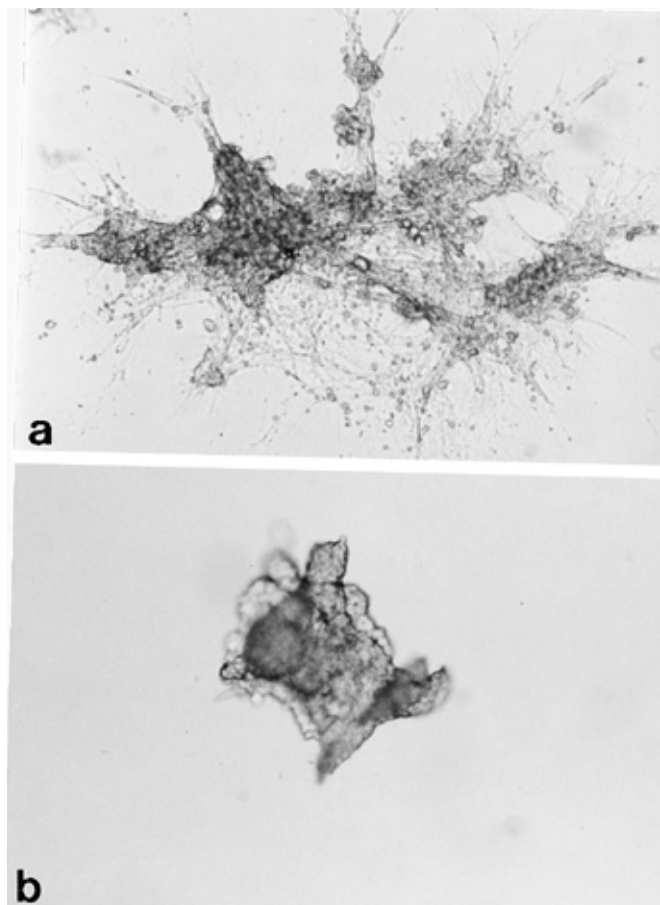


Fig. 1. Light microscopy of: **a**, hydrated whole gluten (sample 1); **b**, starch- and lipid-free gluten (sample 4).

or three components. In analyzing the relaxation data, the number of components that are to be fitted and the estimated  $T_2$  values are supplied before the best fit is found using nonlinear regression methods (Tableware curve fitting software).

### FTIR Experiments

FTIR spectra of samples at 298 K were recorded on a Bio-Rad FTS 60 spectrometer equipped with a HgCdTe detector. A Specac horizontal ATR accessory (Graseby Specac Ltd., Orpington, Kent, UK) with a ZnSe crystal was used to measure the insoluble gluten samples. For all spectra, 256 scans at  $2\text{ cm}^{-1}$  resolution were averaged. The empty ATR crystal was used as reference throughout. Where necessary, traces of water vapor were removed by subtraction of a water vapor spectrum. The protein samples were strewn onto the crystal to cover the whole surface and mixed with an excess of distilled water. This procedure yielded maximally hydrated proteins. The spectra of the hydrated proteins were obtained by subtracting a water spectrum recorded under identical conditions. The criterion for successful water subtraction was a flat baseline at  $2,500\text{--}2,000\text{ cm}^{-1}$ . Distortions from the OH deformation water band are unlikely because no band shift has been reported for water bound to the protein backbone (Careri et al 1979). Afterward, the samples were dried on the crystal in a desiccator over  $\text{P}_2\text{O}_5$  to  $<3\%$  (w/w) water content as determined by Karl-Fischer titration, and the spectra were measured again.

## RESULTS

After freeze-drying, the visual appearance of the gluten samples 1–4 differed according to whether or not starch had been extracted. On the addition of water, samples 1 and 2 formed, as expected, a coherent and viscoelastic mass, but samples 3 and 4 retained loose individual fibers. In light microscope investigations, Amend and Belitz (1991) observed the production of fibrillar strands as hydrated flour or gluten particles were stretched apart either by water surface tension or movement of a coverslip. This they interpreted as strands of the gluten protein polymers, which with further mechanical work, formed thin platelets of protein. The surface of the strands under transmission electron microscope appeared as irregular globules thought to be formed from protein threads strongly twisted and

coiled together. Similar fibrillar strands were formed by samples 1 and 2 on hydration and stretching but not by samples 3 and 4, which remained intact (Fig. 1). Addition of starch to both sets of samples before hydration made no difference to the end result.

Figure 2 shows the FTIR spectra of samples 1–4 in the dry state. There was no difference between the FTIR spectra of samples 1 and 2. In sample 3, a shift of the amide I band maximum from  $1,652$  to  $1,656\text{ cm}^{-1}$  was observed. In sample 4, the amide I band exhibited a maximum at  $1,652\text{ cm}^{-1}$ , but also a prominent shoulder at  $1,625\text{ cm}^{-1}$ , which was not seen in the other samples. No bands arise from starch in these regions. Therefore the changed shape of the amide I bands in 3 and 4 indicate a change in the secondary structure of the protein. The nature of this is difficult to analyze. Bands at  $\approx 1,650$  to  $1,655\text{ cm}^{-1}$  may be assigned to  $\alpha$ -helix and unordered structures, but in gluten proteins there is also a strong contribution at  $1,658\text{ cm}^{-1}$  from glutamine side chains (Krull et al 1965). The shoulder at  $1,625\text{ cm}^{-1}$  has been assigned to  $\beta$ -sheet structures (Pézolet et al 1992).

The distinction between starch-containing and starch-free samples became more pronounced when the proteins were hydrated. Figure 3 shows the FTIR spectra of the hydrated samples. Addition of water to samples 1 and 2 resulted in a limited increase of a shoulder at  $1,620\text{ cm}^{-1}$ , while in sample 3, the shoulder at  $1,619\text{ cm}^{-1}$  was markedly stronger. In sample 4, this increased band overlapped with the strong  $\beta$ -sheet peak at  $1,630\text{ cm}^{-1}$ , making the shoulder very prominent. The  $1,620\text{ cm}^{-1}$  band has been assigned to hydrated extended chains in prolamin (Belton et al 1995), experiencing a solution-like local environment although the samples were insoluble as a whole. Consequently, an increase in this band indicated a more hydrated structure. Comparison of these four samples showed that the starch extraction not only caused changes in the secondary structure of the protein, but the resulting samples were much more accessible to hydration than native or lipid-free samples.

$^2\text{H}$  NMR transverse relaxation in samples 1–4 was multi-exponential and could be represented by a three-component decay: a component with a short  $T_2$  value (short component); an intermediate  $T_2$  value (intermediate component); and a long  $T_2$  value (long component). In all samples at 278 K (Figs. 4–11), the majority of the relaxation ( $\approx 80\%$ ) could be described by the long component with a  $T_2$  value of  $\approx 200$  msec. The difference in  $T_2$

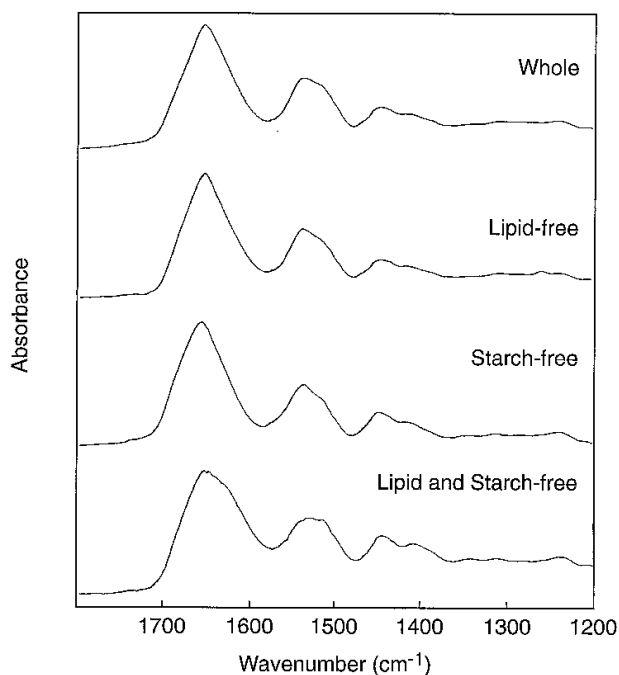


Fig. 2. Fourier transform infrared spectra of dry gluten samples.

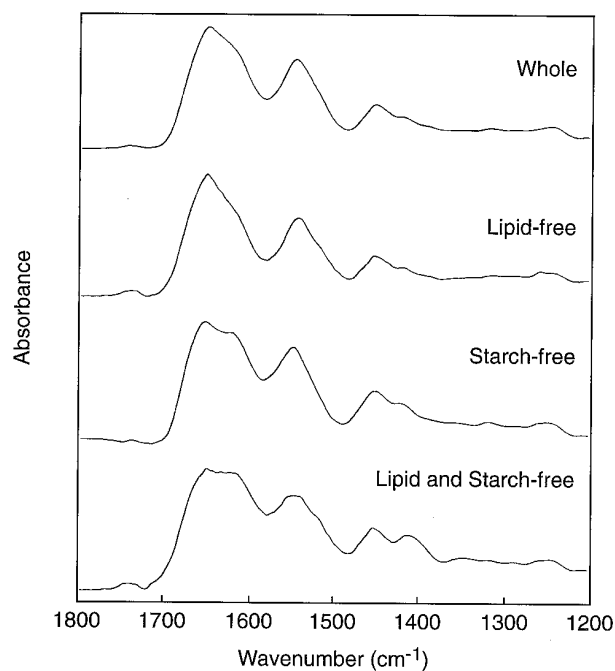


Fig. 3. Fourier transform infrared spectra of hydrated gluten samples.

values of the short and intermediate components was small. The effect of increasing temperature to a maximum of 363 K resulted in increases in the  $T_2$  values of all components, increases in the populations of the short and intermediate components, and a corresponding decrease in the long component.

However, despite these similarities, differences between samples containing residual starch and starch-free samples did exist, the largest being the decrease in the population of the long component which occurred with increasing temperature. This was much more evident in samples 3 and 4 (Figs. 9 and 11) than in samples 1 and 2 (Figs. 5 and 7). Decreases were also more linear than in samples 1 and 2 (Figs. 5 and 7), where more rapid change occurred with increases in temperatures >313 K. As has been discussed previously (Belton et al 1995), the long component can be identified with the bulk  $D_2O$  and a decrease in the percentage of this component represents an increase in the amount of water interacting with the protein. Over the temperature range 278–363 K, samples 3 and 4 absorbed twice as much water as samples 1 and 2 (Table III). Some smaller differences also occurred. The  $T_2$  values of the long component in samples 1 and 2, over the temperature range used, were similar to those of pure  $D_2O$  (Figs. 4 and 6), while those of sample 3 and 4 were slightly lower (Figs. 8 and 10). Populations of the short and intermediate components also differed between samples. These results would indicate that hydration in the starch-containing and starch-free samples differed, due possibly to the methods used to prepare the starch-free samples. To establish whether this was indeed the case, gluten samples that contained both protein and starch and had been subjected to the starch extraction procedures, were prepared. However, these preparations were unsatisfactory in that they produced, on freeze-drying, a separated system of starch with an outer coating of protein.

Because of these problems, it was not possible to obtain information on the effect of starch on the gluten matrix by the analysis of the starch-free samples. However, determination of the hydrophilic nature of the gluten protein was important because the observed decrease in the population of the long component, which occurred on heating samples 1 and 2, may be due in part to sorption of water by the residual starch. Therefore, efforts were made to obtain the water absorption capability of gluten protein by the measurement of the relaxation properties of whole gluten samples containing added starch. To allow straight-forward comparison of the data, the short and intermediate components were co-added to give single

**TABLE III**  
Effect of Temperature on Population of Long Transverse Relaxation ( $T_2$ ) Component of Gluten Samples

Sample	Population (%)			Sorption Rate <sup>b</sup>
	278 K	363 K	Ratio <sup>a</sup>	
1	80	60	1.33	0.0141
2	81	58	1.40	0.0162
3	82	31	2.65	0.0359
4	90	44	2.05	0.0325

<sup>a</sup> Population % at 278 K/population % at 363 K.

<sup>b</sup> Reduction in amount of water not associated with protein, calculated as: g of  $D_2O$ /g of sample/K.

**TABLE IV**  
Effect of Temperature on Population of Long Transverse Relaxation ( $T_2$ ) Component of Gluten Samples With and Without Added Starch

Sample	Population (%)			Sorption Rate <sup>b</sup>
	298 K	363 K	Ratio <sup>a</sup>	
1	72	57	1.26	0.0138
1a	69	52	1.33	0.0159
1b	66	42	1.57	0.0185

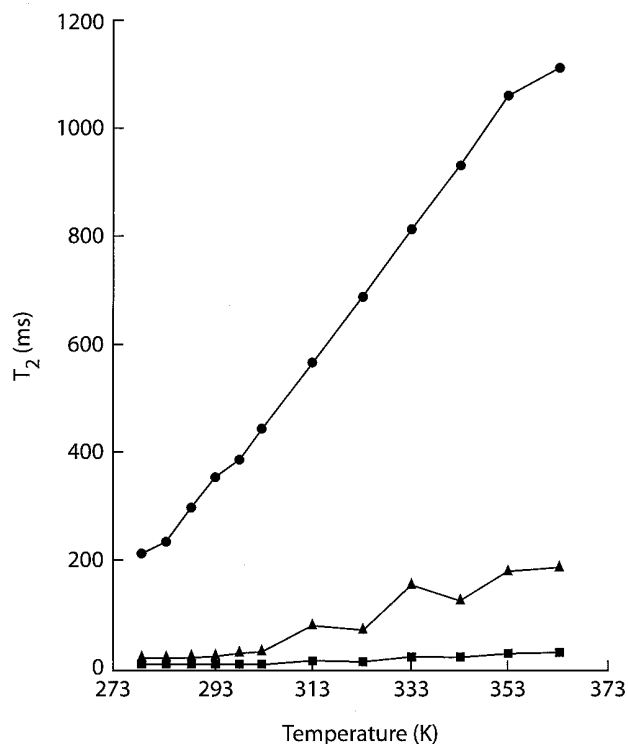
<sup>a</sup> Population % at 298 K/population % at 363 K.

<sup>b</sup> Reduction in amount of water not associated with protein, calculated as: g of  $D_2O$ /g of sample/K.

parameters for  $T_2$  and population. Relaxation was therefore described by a shorter  $T_2$  component (short component) and a longer  $T_2$  component (long component) (Figs. 12–14).

The effects of starch gelation on the relaxation properties of water are clearly demonstrated in the heating of the starch-only sample. Initially, heating produced linear increases in the  $T_2$  values of the short component (Fig. 12) and of the long component (Fig. 13). However, increases in temperature >328 K, which is similar to the reported temperature at which gelation begins (Eliasson 1980, van den Berg 1981), produced accelerated increases in the  $T_2$  values of the short component and sharp decreases in those of the long component. The percentages of component 2 also decreased (Fig. 14) with corresponding increases in component 1, and at 353 K, relaxation became uni-exponential. This is consistent with the process of gelation where starch granules absorb water, and starch fractions dissolve out of the swelled starch granule into the bulk water.

Similar but less dramatic changes are seen in samples 1, 1a, and 1b with  $T_2$  values and populations of the short component tending to increase more rapidly with increases in temperature >328 K (Figs. 12–14). At temperatures <328 K,  $T_2$  values of the short component are larger, and the long component are lower than that of the starch-only sample, indicating greater diffusive exchange (Belton 1990) between the different regions of these samples due to the greater dispersion of the solid in the  $D_2O$ . Using the long component population decrease over the range of 298–363 K, which is greatest in samples containing the highest concentration of starch, it is possible to estimate the amount of water that becomes more closely associated with gluten protein on heating (Table IV), assuming there is no difference between the properties of the residual and added starch. The results suggest that sample 1b absorbs  $\approx 4.7 \times 10^{-3}$  g of  $D_2O$ /g of sample/K more than sample 1. Assuming that this increased value is due totally to the added starch, then the starch content of sample 1 (15% or 3.75 mg), would account for an intake of  $\approx 3.5 \times 10^{-3}$  g of  $D_2O$ /g of sample/K. The amount absorbed by the protein and lipid fraction of the gluten is thus  $12.1 \times 10^{-3}$  g of  $D_2O$ /g of protein and lipid/K at 298 K. The



**Fig. 4.** Effect of temperature on the  $^2H$  transverse relaxation ( $T_2$ ) of  $D_2O$  hydrated whole gluten (sample 1). Short (■), intermediate (▲), and long (●)  $T_2$  components.

total water content of the gluten protein therefore increases from  $\approx 1.7$  g of  $D_2O/g$  of protein and lipid at 298 K to  $\approx 2.6$  g of  $D_2O/g$  of protein and lipid at 363 K. This corresponds to a rate of 0.011 g of  $D_2O/g$  of protein/K. Data from the hydrophobic protein elastin shows a decrease of  $\approx 0.8$  g of  $D_2O/g$  of protein to  $\approx 0.2$  g of  $D_2O/g$  of protein over a temperature range of 280–335 K (Ellis and Packer 1976). The difference between the hydrophilic gluten and the hydrophobic elastin is thus sharply contrasted (Belton et al 1994).

It may be stated that the method of preparation of the starch-added samples was not a wholly intimate mixture, and few conclusions could be drawn as to the effect of the starch on the gluten matrix. However, it is likely that absorption of water by the added starch during gelation is no less than that of residual starch (Eliasson 1983), which is more closely associated with the protein and lipid. The calculated value of water absorbed by the protein-lipid of the gluten, therefore, may be a conservative estimate.

### DISCUSSION

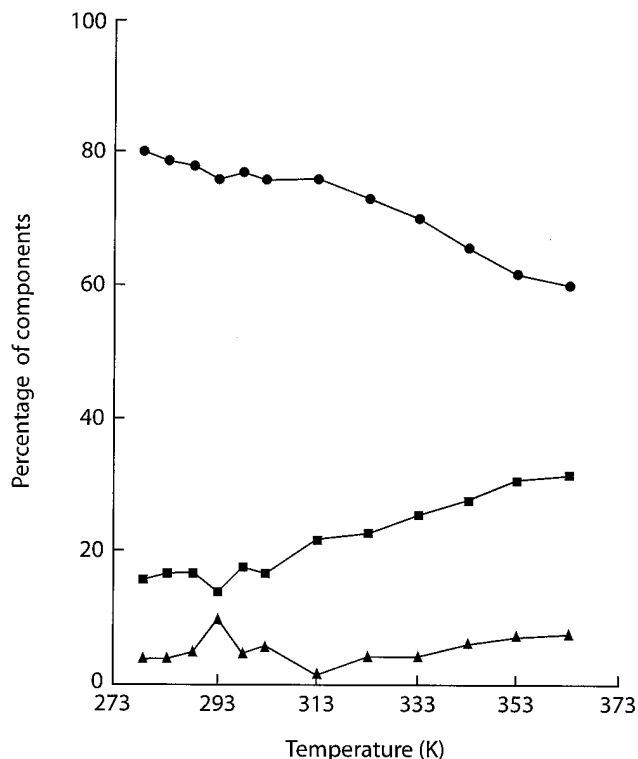
Multieponential transverse relaxation has been previously observed in similar experiments on high  $M_r$  subunits (Belton et al 1994). In a hydrated system, we expect there to be a diffusive exchange between bulk water and water more intimately associated with the biopolymer phase (Belton 1990). There will also be chemical exchange between exchangeable protons or deuterons on the biopolymers and water. Such a system is expected to exhibit multiple relaxation processes and, in general, the time constants and populations will be pulse-spacing dependent (Belton 1990). The slowest decaying fraction of the transverse relaxation process had relaxation times close to those for pure  $D_2O$ , and may be identified with the bulk water phase. Typically, for a water diffusion coefficient of  $2.5 \times 10^{-9}$  m<sup>2</sup>/sec, the root mean square displacement on a timescale of 1 sec would be 60  $\mu$ m, which is small compared to the dimensions of the aqueous phase in a system where there is an observable supernatant phase (as was the case for the starch-containing samples). In the case of the more dispersed samples, no

clear supernatant was observed. However, the relaxation times of the slowest decaying components were also close to that of bulk water and may therefore be identified with water that is not in close proximity to the protein matrix.

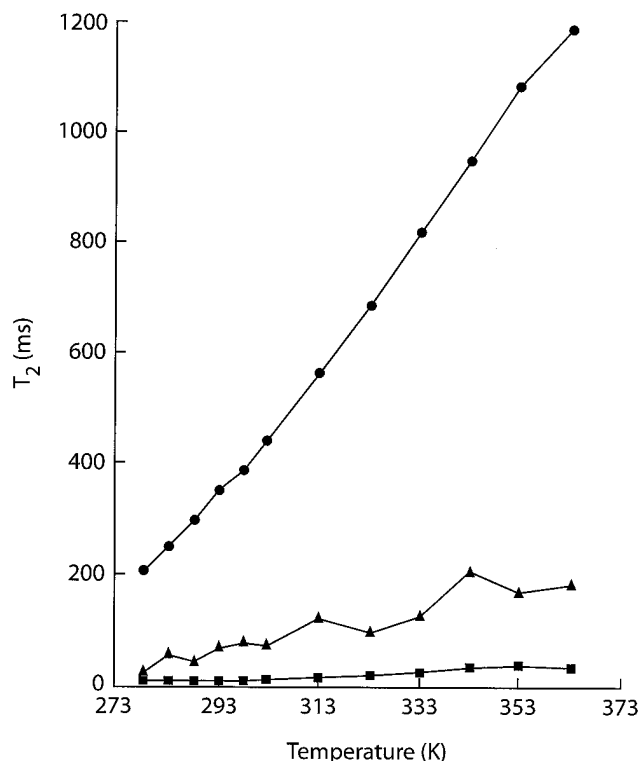
In general, it is expected that a hydrophilic matrix will expand and absorb water on heating, whereas a hydrophobic one will contract and expel water (Ellis and Packer 1976, Belton et al 1994). On heating the whole gluten with excess water, the bulk water fraction shows a smooth increase in relaxation time and a smooth decrease in relative proportion. This shows that the gluten mass has a positive coefficient of expansion, consistent with a hydrophilic character. As the mass is heated, it expands and absorbs more water. The remaining two components show relatively little change in rate and an increase in proportion consistent with the loss in the bulk fraction.

Extraction of lipid makes very little difference to the hydration behavior of the gluten on heating, indicating that the lipid neither has much interaction with the water nor does it inhibit the interaction of the protein with the water. There is no evidence, therefore, that the lipid might form a protective hydrophobic coating to the protein. The results may support the view that much of the lipid is organized into phase structures such as liposomes or vesicles that are retained within the gluten in a fairly nonspecific way (Carr et al 1992, Carcea and Schofield 1996). Lipid binding may then occur either by physical entrapment within the protein matrix or by bonding between protein and phase surfaces (Carr 1991). If the latter is the case, then it must be concluded that the interfacial area affected by the interaction with the lipid phase surface must be small, otherwise a significant effect of lipid removal would be observed. However, the dramatic effects of dissolution and freeze-drying of the gluten matrix in the starch removal process shows that, once seriously disrupted, the original equilibrium is not restored.

Unlike lipid, dry wheat starch granules are highly hydrophilic, and when suspended in excess water at ambient temperatures, the granules will take up water. Swelling, as measured by increases in granule diameter, may be of the order of 30% (van den Berg 1981).



**Fig. 5.** Effect of temperature on the  $^2H$  transverse relaxation ( $T_2$  component population) of  $D_2O$  hydrated whole gluten (sample 1). Short (■), intermediate (▲), and long (●)  $T_2$  components.



**Fig. 6.** Effect of temperature on the  $^2H$  transverse relaxation ( $T_2$ ) of  $D_2O$  hydrated lipid-free gluten (sample 2). Short (■), intermediate (▲), and long (●)  $T_2$  components.

Swelling is much greater during gelation, where the first stages occur over the temperature range of 323–334 K, and granules may absorb as much as 40× their weight of water (van den Berg 1981). The contribution of the residual starch was estimated by adding

starch to intact gluten, but even if the effect of the residual starch is taken into account, the proteins still absorb water on heating. Moreover, the samples with residual starch extracted were actually more hydrophilic than the native gluten.

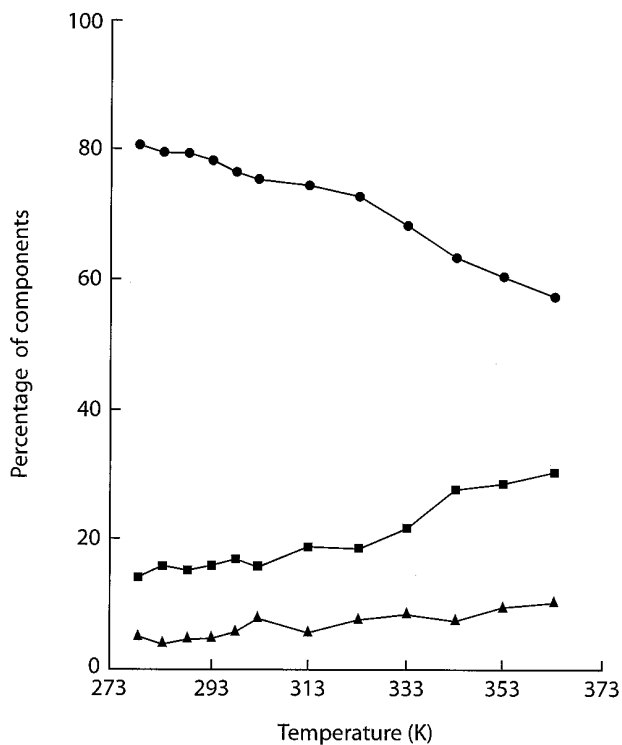


Fig. 7. Effect of temperature on the  $^2\text{H}$  transverse relaxation ( $T_2$  component population) of  $\text{D}_2\text{O}$  hydrated lipid-free gluten (sample 2). Short (■), intermediate (▲), and long (●)  $T_2$  components.

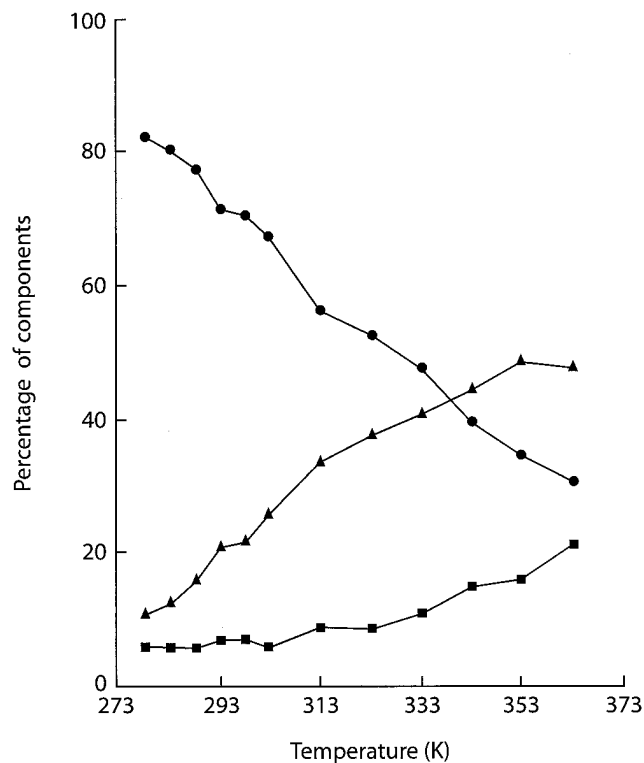


Fig. 9. Effect of temperature on the  $^2\text{H}$  transverse relaxation ( $T_2$  component population) of  $\text{D}_2\text{O}$  hydrated starch-free gluten (sample 3). Short (■), intermediate (▲), and long (●)  $T_2$  components.

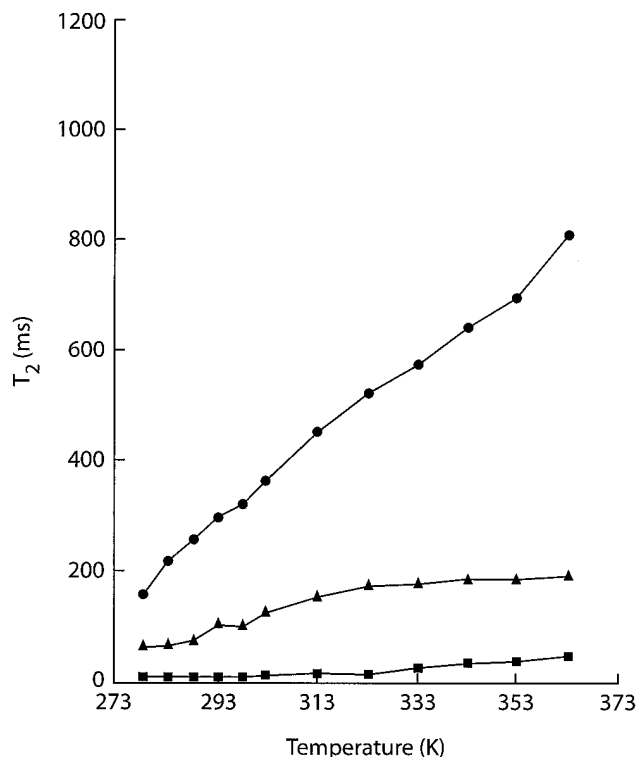


Fig. 8. Effect of temperature on the  $^2\text{H}$  transverse relaxation ( $T_2$ ) of  $\text{D}_2\text{O}$  hydrated starch-free gluten (sample 3). Short (■), intermediate (▲), and long (●)  $T_2$  components.

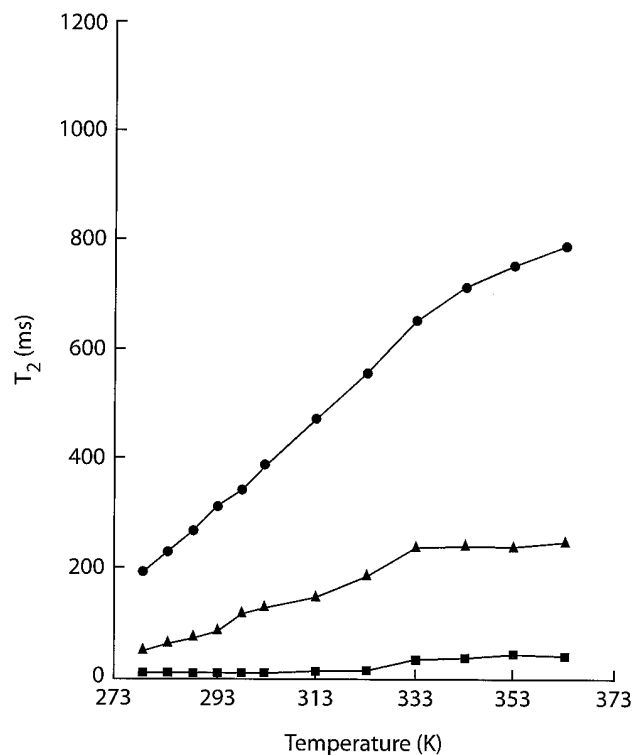
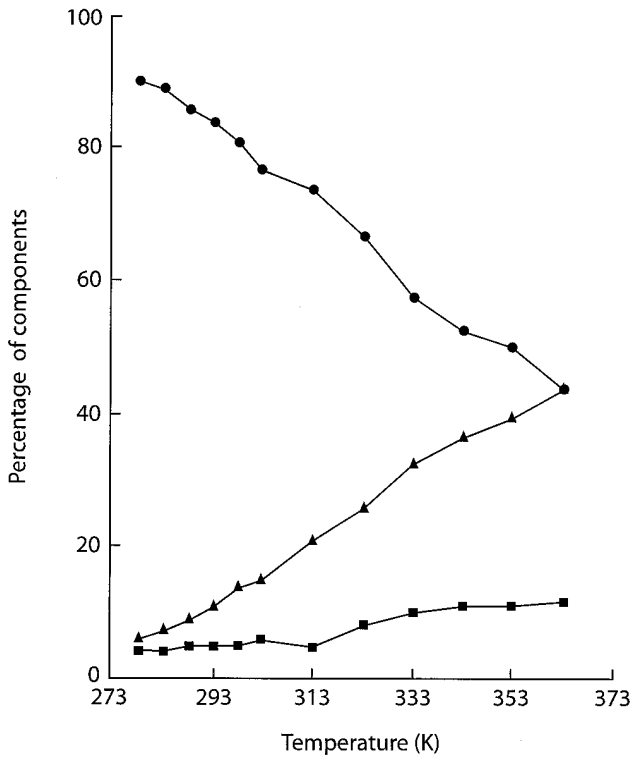
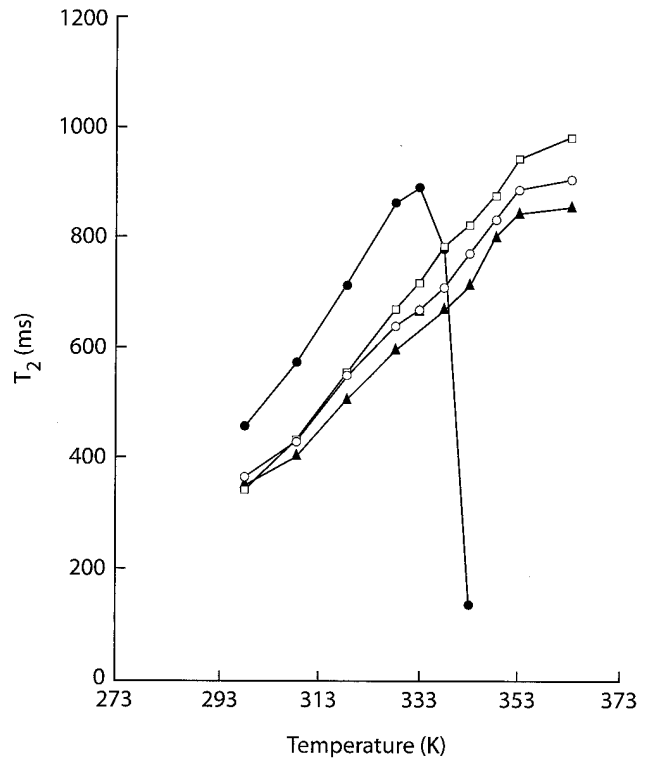


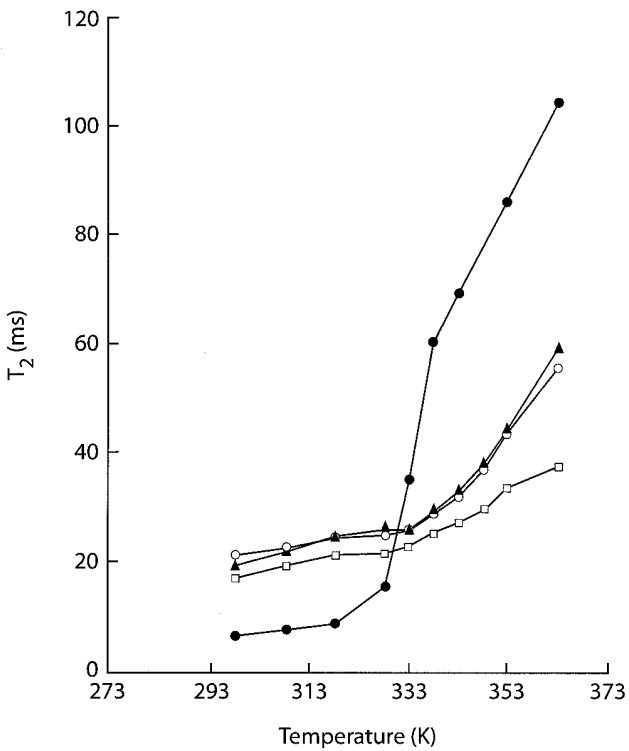
Fig. 10. Effect of temperature on the  $^2\text{H}$  transverse relaxation ( $T_2$ ) of  $\text{D}_2\text{O}$  hydrated starch- and lipid-free gluten (sample 4). Short (■), intermediate (▲), and long (●)  $T_2$  components.



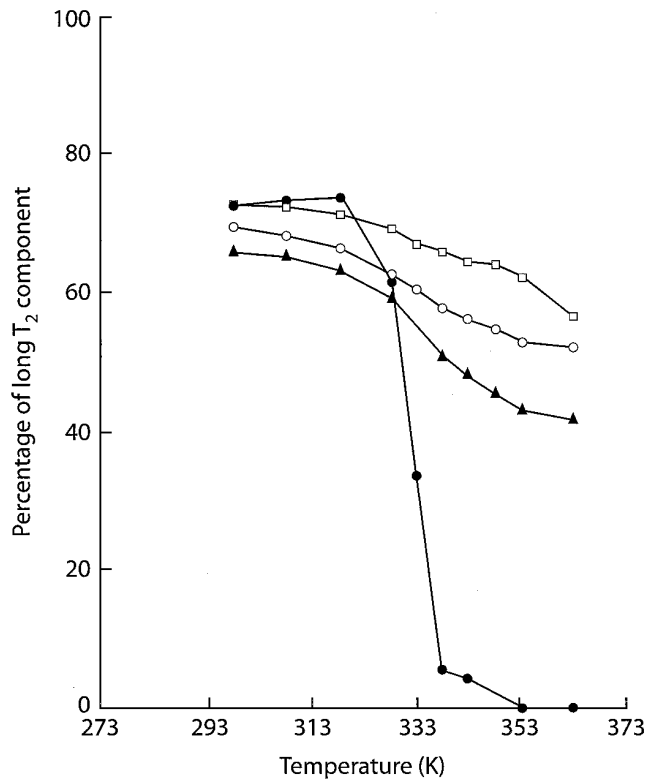
**Fig. 11.** Effect of temperature on the  $^2\text{H}$  transverse relaxation ( $T_2$  component population) of  $\text{D}_2\text{O}$  hydrated starch- and lipid-free gluten (sample 4). Short ( $\blacksquare$ ), intermediate ( $\blacktriangle$ ), and long ( $\bullet$ )  $T_2$  components.



**Fig. 13.** Effect of temperature on the  $^2\text{H}$  transverse relaxation (long  $T_2$ ) of  $\text{D}_2\text{O}$  hydrated samples. Starch ( $\bullet$ ), whole gluten ( $\square$ ), whole gluten with 1.4% (w/w) added starch ( $\circ$ ), whole gluten with 2.8% (w/w) added starch ( $\blacktriangle$ ). Control and samples 1, 1a, and 1b, respectively.



**Fig. 12.** Effect of temperature on the  $^2\text{H}$  transverse relaxation (short  $T_2$ ) of  $\text{D}_2\text{O}$  hydrated samples. Starch ( $\bullet$ ), whole gluten ( $\square$ ), whole gluten with 1.4% (w/w) added starch ( $\circ$ ), whole gluten with 2.8% (w/w) added starch ( $\blacktriangle$ ). Control and samples 1, 1a, and 1b, respectively.



**Fig. 14.** Effect of temperature on the  $^2\text{H}$  transverse relaxation (long  $T_2$  component population) of  $\text{D}_2\text{O}$  hydrated samples. Starch ( $\bullet$ ), whole gluten ( $\square$ ), whole gluten with 1.4% (w/w) added starch ( $\circ$ ), whole gluten with 2.8% (w/w) added starch ( $\blacktriangle$ ). Control and samples 1, 1a, and 1b, respectively.

This surprising result is almost certainly due to the starch removal process that requires major disruption of disulfide linkages and the dissolution of the polymers. It seems unlikely that the intermolecular interactions will be restored in their entirety by freeze-drying and subsequent rehydration. The results of the light microscopy suggest that the close-knit structure of the protein threads may have been destroyed, resulting in the inability of the starch-free samples to form fibrillar strands of gluten on hydration and stretching. The evidence from both NMR and FTIR is that the altered structure of the starch-free samples is more accessible to water. Evidently the disruption of the structure results in fewer protein-protein interactions and thus a greater tendency to swell and absorb water than in the native material.

It is suggested, therefore, that the overall balance of the properties of the mixture of proteins that constitute gluten is hydrophilic rather than hydrophobic. The insolubility of the gluten mass, therefore, does not reside in intrinsic hydrophobicity but must reside in interchain entanglement mechanisms. These findings are contrary to those of van Dijk et al (1998), who reported that the solubility of Dx5, a high molecular subunit of wheat glutenin, was determined by the hydrophobic N-terminal A-domain. However, the domain construct investigated contained a hydrophobic signal sequence that may have affected results.

Gluten proteins contain many inter- and intramolecular disulfide bonds, and there are many noncovalent interchain interactions by hydrogen bonding of glutamine residues. A mechanism has been proposed that explains the observed hydration characteristics (Belton 1994, Belton et al 1995). On hydration, glutamine-glutamine hydrogen bonds are broken and glutamine-water hydrogen bonds are formed. However, even in parts where there are no disulfide bonds, statistics dictate that sufficient interchain bonds remain to ensure that there is not complete dissociation. This is the "loop and train" model (Doi and Edwards 1986, Belton et al 1995). The effect of heating will be to break more interchain hydrogen bonds. This will result in sorption of additional water to hydrate the released polar groups.

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#### LITERATURE CITED

- Amend, T., and Belitz, H.-D. 1991. Microstructural studies of gluten and a hypothesis on dough formation. *Food Structure* 10:277-288.
- Belton, P. S. 1990. Can nuclear magnetic resonance give useful information about the state of water in foodstuffs? *Comments Agric. Food Chem.* 2:179-209.
- Belton, P. S. 1994. A hypothesis concerning the elasticity of high molecular weight subunits. Pages 159-165 in: *Proceedings of the International Meeting on Wheat Kernel Proteins, Molecular and Functional Aspects.* Università Degli Studi Della Tuscia. S. Martino al Cimino: Viterbo, Italy.
- Belton, P. S., Colquhoun, I. J., Field, J. M., Grant, A., Shewry, P. R., and Tatham, A. S. 1994. <sup>1</sup>H and <sup>2</sup>H NMR relaxation studies of a high  $M_r$  subunit of wheat glutenin and comparison with elastin. *J. Cereal Sci.* 19:115-121.
- Belton, P. S., Colquhoun, I. J., Field, J. M., Grant, A., Shewry, P. R., Tatham, A., and Wellner N. 1995. FTIR and NMR studies on the hydration of a high  $M_r$  subunit of glutenin. *Int. J. Biol. Macromol.* 17:74-80.
- Carcea, M., and Schofield, J. D. 1996. Protein-lipid interactions in wheat gluten: Reassessment of the occurrence of lipid-mediated aggregation of protein in the gliadin fraction. *J. Cereal Sci.* 24:101-113.
- Careri, G., Giansanti, A., and Gratton, E. 1979. Lysozyme film hydration events: An IR and gravimetric study. *Biopolymers* 18:1187-1203.
- Carr, O. N. 1991. Lipid binding and lipid-protein interactions in wheat flour dough. PhD thesis. University of Reading: Reading, UK.
- Carr, O. N., Daniels, N. W. R., and Frazier, P. J. 1992. Lipid interactions in breadmaking. *Crit. Rev. Food Sci. Nutr.* 31:237-258.
- Chung, O. K. 1986. Lipid-protein interactions in wheat flour dough, gluten, and protein fractions. *Cereals Food World* 31:242-256.
- Colquhoun, I. J., and Goodfellow, B. J. 1994. Nuclear magnetic resonance spectroscopy. Pages 87-145 in: *Spectroscopic Techniques for Food Analysis.* R. H. Wilson, ed. VCH Publishers: New York.
- De Stefanis, V. A., and Ponte, J. G., Jr. 1976. Studies on the breadmaking properties of wheat-flour nonpolar lipids. *Cereal Chem.* 53:636-642.
- Doi, M., and Edwards, S. F. 1986. Dynamics of a polymer in a fixed network. Pages 189-217 in: *Theory of Polymer Dynamics.* Int. Series of Monographs on Physics 73. R. K. Adair, R. J. Elliott, J. A. Krumhansl, W. Marshall, and D. H. Wilkinson, eds. Clarendon Press: Oxford, UK.
- Eliasson, A.-C. 1980. Effect of water content on the gelatinization of wheat starch. *Starch/Staerke* 32:270-272.
- Eliasson, A.-C. 1983. Differential scanning calorimetry studies on wheat starch-gluten mixtures. I. The effect of gluten on the gelatinization of wheat starch. *J. Cereal Sci.* 1:199-205.
- Ellis, G. E., and Packer, K. J. 1976. Nuclear spin-relaxation studies of hydrated elastin. *Biopolymers* 15:813-832.
- Eliasson, A.-C., and Tjerneld, E. 1990. Adsorption of wheat proteins on wheat starch granules. *Cereal Chem.* 67:366-372.
- Genot, C., Montenay-Garestier, T., and Drapron, R. 1984. Intrinsic spectrofluorometry applied to soft wheat (*Triticum aestivum*) flour and gluten to study lipid-protein interactions. *Lebensm. Wiss. Technol.* 17:129-133.
- Krull, L. H., Wall, J. S., Zobel, H., and Dimler, R. J. 1965. Synthetic polypeptides containing side-chain amide groups: Water insoluble polymers. *Biochemistry* 4:626-633.
- MacRitchie, F. 1984. Baking quality of flour. Pages 201-277 in: *Advances in Food Research*, Vol 29. C. O. Chichester, ed. Academic Press: Orlando, FL.
- MacRitchie, F. 1992. Physicochemical properties of wheat proteins in relation to functionality. *Adv. Food Nutr.* 36:1-87.
- Morrison, W. R. 1988. Lipids. Pages 373-439 in: *Wheat Chemistry and Technology*, Vol. 1. Y. Pomeranz, ed. Am. Ass. Cereal Chem.: St. Paul, MN.
- Morrison, W. R., Greenwell, P., Law, C. N., and Sulaiman, B. D. 1992. Occurrence of friabilin, a low molecular weight protein associated with grain softness, on starch granules isolated from some wheats and related species. *J. Cereal Sci.* 15:143-149.
- Nishiyama, J., Kuninori, T., Matsumoto, H., and Hyono, A. 1981. ESR studies on lipid-protein interactions in gluten. *Agric. Biol. Chem.* 45:1953-1958.
- Pézolet, M., Bonenfant, S., Dousseau, F., and Popineau, Y. 1992. Conformation of wheat gluten proteins. Comparison between functional and solution states as determined by infrared spectroscopy. *FEBS Lett.* 299:247-250.
- Tatham, A. S., Shewry P. R., and Belton, P. S. 1990. Structural studies of cereal prolamins, including wheat gluten. Pages 1-78 in: *Advances in Cereal Science and Technology*, Vol. 10. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Weegels, P. L., Hamer, R. J., and Schofield, J. D. 1996. Functional properties of wheat glutenin. *J. Cereal Sci.* 23:1-18.
- Wehrli, H. P., and Pomeranz, Y. 1970. A note on the interaction between glycolipids and wheat flour macromolecules. *Cereal Chem.* 47:160-166.
- van den Berg, C. 1981. Vapour sorption equilibria and other water-starch interactions: A physico-chemical approach. PhD thesis. Department of Food Science, Agricultural University: Wageningen, The Netherlands.
- van Dijk, A. A., van Swieten, A. A., Kruize, I. T., and Robillard, G. T. 1998. Physical characterisation of the n-terminal domain of high-molecular-weight glutenin subunit Dx5 from wheat. *J. Cereal Sci.* 28:115-126.
- Zawistowska, U., Bekes F., and Bushuk, W. 1985a. Gluten proteins with high affinity to flour lipids. *Cereal Chem.* 62:284-289.
- Zawistowska, U., Bekes, F., and Bushuk, W. 1985b. Involvement of carbohydrates and lipids in aggregation of glutenin proteins. *Cereal Chem.* 62:340-345.

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