Water binding to biopolymers in different cereals and legumes: proton NMR relaxation, dielectric and water imbibition studies

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Proton NMR relaxation time (T_1), dielectric properties by means of the thermally stimulated depolarization currents (TSDC) method, and water imbibition were measured in cereal and legume grains (wheat, triticale, maize, pea, chick pea, horsebean, white lupin, lentil and beans) having different chemical composition (proteins, carbohydrates, lipids). T_1 versus water content in the range 0.05–1.40 g water/g dry matter showed characteristic V-shaped curves with a sharp or a broad minimum depending on the species. Water content at $T_{1\,min}$ was in high positive correlation with protein content of the grains ($r=0.90$) and in high negative correlation with soluble carbohydrates ($r=-0.92$), while lipids gave a very low correlation ($r=0.38$). The water content at $T_{1\,min}$ (0.18–0.47 g water/g dry matter) was assigned to a primary hydration sphere around the macromolecules, since, when T_1 was plotted versus per cent maximum hydration, the $T_{1\,min}$ values for all grains fell between 25–30% of maximum hydration. The extrapolated value for zero protein content was 0.08 g water/g dry matter, which coincided with data in the literature for the water monolayer on starch. The TSDC measurements enabled us to determine the amount of tightly (irrotationally) bound water at primary hydration sites to 0.18 ± 0.02 g water/g dry matter for beans, pea and chickpea, and, tentatively, to about 0.10 g water/g dry matter for wheat. Water imbibition data for 11 cereal and legume species gave total water hydration capacity in the range $a=0.44$–1.82 g water/g dry matter. This value divided by the water content of the primary hydration sphere (swelling index) was also in high positive correlation with the protein content of the grains ($r=0.84$).

1. Introduction

Proton spin–lattice (T_1) and spin–spin relaxation time (T_2) of water are parameters measured by the nuclear magnetic resonance (NMR) technique, which can be related to molecular mobility and therefore give some information on interactions between water molecules and macromolecules in different foods and natural products [1, 2]. The usual way is to study resonance frequency, temperature or water content dependence for any one of these relaxation parameters.

We have compiled some data from the literature on T_1 versus water content in different biological and macromolecular systems, and in all cases characteristic V-shaped curves were obtained, while the value for water content at the T_1 minimum was given in Table I.

Dielectric measurements by different techniques have been used to measure the hydration properties of seeds [9–11]. The dielectric technique of thermally stimulated depolarization currents (TSDC), which corresponds to measuring dielectric losses versus temperature at fixed low frequencies [12] have been shown to be very powerful in determining the fraction of tightly bound water in biological materials [13].

In a previous paper [14] we studied the effect of seed moisture content (5–50%) on T_1 in different maize genotypes by separating the contribution to the NMR signal from seed germ and seed endosperm, and particularly by separating the signals of the germ lipids and water.

This study was undertaken on grains of eight different cereals and legumes (wheat, triticale, maize, pea, cowpea, horsebean, white lupin and lentil) with the intention to measure the complete T_1 versus water content curves (0.05–1.40 g water/g dry matter), and to try to correlate the position of the observed minima with chemical composition of the grains (proteins, carbohydrates, lipids). We assigned the water content

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TABLE I Water content at $T_{1\,min}$ obtained from proton NMR measurements on different macromolecular and biological systems as compiled from literature

Sample	Water content at $T_{1\,min}$ (g water/g dry matter)	ν_0 (MHz)	References
Cellulose[a]	0.09	60	[3]
Corn Starch	0.15	10	[4]
Casein	0.17	10	[4]
Pectin	0.22	10	[4]
Sodium alginate	0.26	10	[4]
Myosine	0.227	90	[5]
Soy flour[b]	0.387	7	[6]
Wheat seed	0.18	60	[7]
Artemia cyst	0.25	–	[8]

[a] Position of $T_{1\,min}$ for six different types of cellulose ranging between 0.053–0.136 g water/g dry matter.
[b] "Bound" water content obtained with a wide line NMR spectrometer.

at $T_{1\,min}$ to a primary hydration sphere around macromolecules, while the extrapolation of the regression line $T_{1\,min}$ versus protein content for zero proteins coincided with the water monolayer for starch.

TSDC measurements were carried out on compressed pellets of ground seeds of beans, pea, chickpea and wheat in the temperature range 77–300 K and the water content range 0.02–0.25 g water/g dry matter. For water contents higher than a critical value a new relaxation appears which is attributed to the reorientation of water molecules in frozen water clusters around the primary hydration sites. The measurements allow this critical water content, which corresponds to the amount of tightly (irrotationally) bound water to be determined.

Additionally studies were undertaken on water imbibition for 11 cereals and legumes to obtain total water hydration capacity and the correlation between total hydration capacity/primary hydration sphere and protein content.

2. Materials and methods

2.1. Moisture conditioning

Conditioning of moisture contents of cereal and legume grains for NMR measurement in the range between 0.05–1.40 g water/g dry matter was done by two procedures: (a) hydration of grains in desiccators with distilled water and subsequent dehydration in desiccators with $CaCl_2$, and (b) imbibition of grains in water and continual dehydration with $CaCl_2$ over many days.

The water content of the grains was continually measured during the dehydration procedure and the final dry weight was estimated by drying at 105–110 °C. Water content was expressed either as a percentage or as grammes water per gramme dry weight of the grain.

For TSDC measurements the seeds were uniformly ground, the powder compressed at 6 tons into cylindrical pellets of 13 mm diameter and about 1.5 mm height. The density of the dry pellets was 1.10 ± 0.02 g cm^{-3} for wheat and 1.16 ± 0.02 g cm^{-3} for beans, pea and chickpea. The water content was varied between 0.02 and 0.25 g water/g dry matter by equilibrating the samples prior to dielectric measurements, in closed jars over saturated salt solutions [9]. Drying at 110 °C and 6.665 Pa for 24 h was adopted for the determination of dry weights.

2.2. NMR measurements

The proton spin–lattice relaxation time (T_1) was measured with a pulsed NMR relaxometer (model IJS-2-71 modified) using a 90°–τ–90° pulse sequence [15], and usually at the resonance frequency 32 MHz for single kernel analysis (diameter of the radio frequency coil 12 mm). In some cases when bulk samples were used, the operational frequency was 16 MHz enabling larger samples to be run (diameter 24 mm). More details on T_1 measurement are given elsewhere [14, 16].

Each T_1 versus water content curve is a collection of data for at least 3–5 individual grains in the whole moisture content (5–60%) range. T_1 data for dehydration only were taken into account, since in most cases a hysteresis in T_1 was obtained for the hydration/dehydration cycle. This means that T_1 was usually higher during hydration (more free water with longer T_1) than during dehydration when there has been enough time for the water molecules to diffuse deeper into the grain and to interact with macromolecules giving a somewhat shorter T_1.

2.3. TSDC measurements

The TSDC method consists of measuring the thermally activated release of stored dielectric polarization [12]. The sample is polarized by a d.c. electric field and then cooled down to a sufficiently low temperature (in our case liquid nitrogen temperature) to freeze-in the polarization. The field is then switched off and the sample is warmed up at a constant rate while the depolarization current, as the dipoles relax, is measured. Thus for each polarization mechanism an inherent current peak can be detected. The theory, the apparatus and the procedures used to determine the parameters characterizing the dielectric behaviour of a sample have been described elsewhere [12, 13].

2.4. Water imbibition and hydration capacity

Water imbibition during a period of ≈30 h was measured for individual grains (4–16) at 0.5–1 h time intervals, and the average hydration capacity for each species was calculated according to a model given by Blacklow [17] and later applied by us to imbibition of different maize genotypes [18]. The uptake of water was calculated on a dry matter basis after drying (overnight at 105 °C) at the end of imbibition ($t = 31$ h) and the following equation was applied to calculate hydration capacity a:

$$W_t = a + bt - (a - W_0) \exp(-Kt) \quad (1)$$

In this equation W_0 and W_t are initial water content and water content at moment t (dry weight basis), respectively, K (h^{-1}) is the initial water uptake rate, and b (% h^{-1}) represents water uptake in the later stage of imbibition.

3. Results and discussion

3.1. Water content at $T_{1\,min}$

Data in the literature on T_1 NMR measurement of a few macromolecular systems containing water show that the water content at T_{1min} ranges from an average 0.09 g water/g dry matter in cellulose up to 0.387 g water/g dry matter in soy flour (Table I). According to these results as well as to our own measurements on cereals and legumes it seems that the position of T_{1min} is independent of the resonance frequency $\nu = \omega_0/2\pi$(ω_0 is the angular frequency) in the range 7–60 MHz, but is rather dependent on the chemical composition of the sample, particularly regarding the protein content.

T_1 versus water content curves were measured for eight different species and the resulting patterns for four of them (wheat, triticale, pea and horsebean) are shown in Fig. 1. The water content was changed from about 5% up to almost 60% (wet weight basis), and the values of water content at T_{1min} expressed as g water/g matter are shown in Table II for all species investigated. They ranged from 0.18–0.41 g water/g dry matter, which is in agreement with data in the literature for the other systems studied (Table I).

There are several possible interpretations of the V-shaped curves obtained for T_1 versus water content in different cereal and legume seeds. The familiar relation between T_1 of protons in H_2O and the correlation time τ_c, which is a parameter of molecular mobility, is given by the equation [19]:

$$\frac{1}{T_1} = \frac{3}{10}\frac{\gamma_H{}^2\hbar^2}{r^6}\left[\frac{\tau}{1+\omega^2\tau_c^2} + \frac{4\tau_c}{1+4\omega^2\tau_c^2}\right] \quad (2)$$

where γ_H is the gyromagnetic constant for protons, and $\hbar$ is Planck's constant divided by 2π. The situation is complicated even in pure water because one has to take into account both intramolecular and intermolecular contribution to the relaxation, and also the translational and rotational motion of the molecule are coupled together to give molecular reorientation. The theoretical dependence between T_1 and τ_c for a water molecule is a V-shaped curve which takes into account the experimental fact that T_1 in bulk water (short $\tau_c \approx 10^{-12}$ s) is similar to T_1 in ice (long $\tau_c \approx 10^{-5}$ s).

Additional complications arise when one tries to interpret relaxation data for water adsorbed on macromolecules. A distribution of correlation times of water molecules near surfaces was postulated to obtain better fitting of experimental data to the theory [20]. According to such interpretations, which did not take into account magnetic interactions or chemical exchange between water and macromolecular protons, the longer T_1 values measured in seed with low water content should correspond to water bound to macromolecules of the seed matrix, with $\tau_c \approx 10^{-5}$ s, i.e. like in ice. When water content is increased T_1 passes through the minimum and then rises again since more and more water molecules are reorienting with τ_c close to that of bulk water.

More recent NMR experiments revealed that intermolecular exchange and cross-relaxation are the dominant mechanisms in water–protein systems [21, 22], and this model did not assume the existence of bound water at the protein surface with long τ_c, and particularly not having the properties of ice. Instead this model assumes a relatively fluid water hydration layer around a protein molecule and chemical or

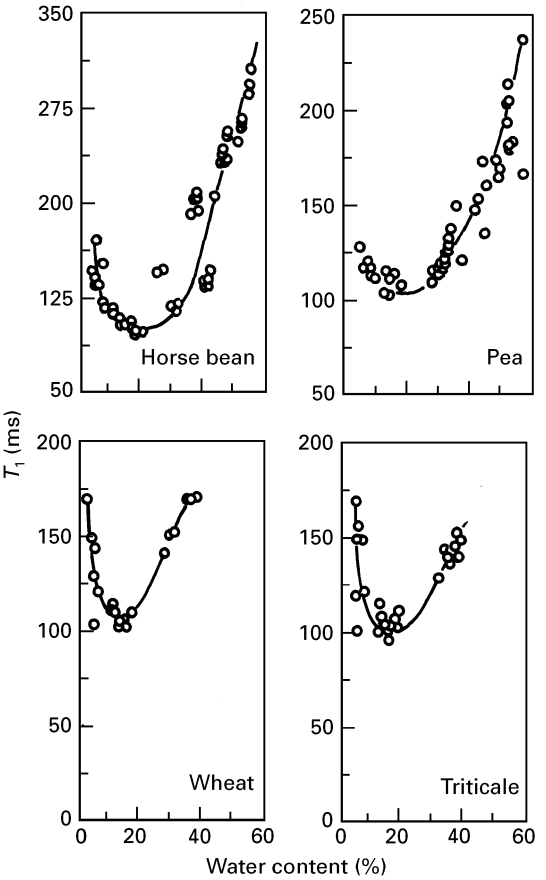

Figure 1 Spin-lattice relaxation time (T_1) of water protons versus water content (%) of the grains of two cereals (wheat, triticale) and two legumes (pea, horsebean). The curves drawn are a guide for the eye only. The points for each species were collected from measurements on a few individual grains.

TABLE II Water content at $T_{1\,min}$ for different cereal and legume seeds

Variety	Water content at T_{1min} (g/g)
Wheat	0.18
Triticale	0.19
Maize	0.18[a]
Horsebean	0.28
Pea	0.28
Cowpea	0.35
White lupine	0.39
Lens	0.41

[a] Taken from Ratković [14].

spin exchange between H-atoms from water and the protein.

We have assumed that the water content at $T_{1\,min}$ in seed corresponded to the primary hydration sphere around the macromolecules, and that its values are between 0.18 and 0.41 g water/g dry seed depending on the genotype (Table II). An average correlation time τ_c of water molecules in the primary hydration sphere would be not longer than 10^{-9}–10^{-8} s. When the water content of a seed is further increased, T_1 also increases (Fig. 1) reflecting the composite behaviour of water + macromolecules, i.e. plasticization of proteins by surrounding water molecules produces more motional freedom in the whole system, and therefore a further decrease in τ_c. At the very high water contents obtained during imbibition there are probably water fractions in the seed (trapped water or some interfacial water) with a correlation time as short as that for bulk water ($\approx 10^{-12}$ s). Below $T_{1\,min}$ T_1 increases again (Fig. 1) since at low water content cross-relaxation and spin exchange become the dominant phenomena. The hydrogen spins of water molecules in a monolayer around a protein molecule will exchange with protein hydrogens, which have a long T_1 as in most solids, and the resulting T_1 will increase compared with the value at the minimum.

An alternative explanation for increased T_1 at low water content in seeds comes from the so called water replacement hypothesis [8, 23] which was developed to account for the survival of dry organisms under conditions of extreme desiccation. The point is that substances containing hydroxyl groups, like glycerol, trehalose or sugar alcohols can replace water molecules close to the macromolecular or membrane surface stabilizing these structures in conditions of extreme dehydration. The water molecules so released will have τ_c similar to that of bulk water and the resulting T_1 will be long.

Based on the measured data, we supposed that the water content at $T_{1\,min}$ corresponded to a primary hydration sphere around the macromolecules, which changes between 0.18 and 0.41 in different grains depending on protein content (Table II). When this was plotted against protein content a linear regression

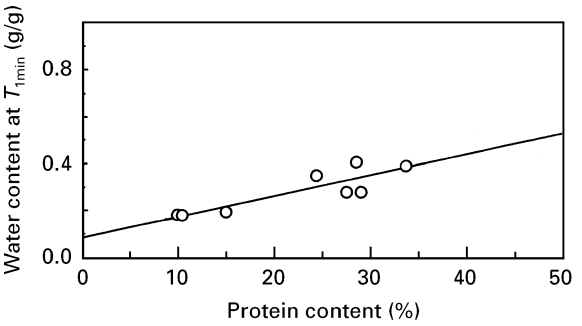

Figure 2 Water content at $T_{1\,min}$ versus protein content for eight cereals and legumes ($r = 0.90$).

with a high correlation of $r = 0.90$ was obtained, and the extrapolation for zero protein content gave a water content around 0.08 g water/g dry matter (Fig. 2). This could represent the water monolayer around starch, since it is in close agreement with the values obtained for maize starch (0.075 g/g) and potato starch (0.08 g/g), while the water monolayer values for absorption on specific sites was 0.07 ± 0.01 g/g [24].

Due to the negative correlation between protein and soluble carbohydrate contents (Table III), the correlation between the water content at $T_{1\,min}$ and soluble carbohydrates is also high ($r = -0.92$). As expected, the correlation between water content at $T_{1\,min}$ and content of lipids was very low ($r = 0.38$).

The observed high positive correlation between size of the primary hydration shell and protein content for cereals and legumes is in agreement with studies of water sorption on isolated proteins [28–31]. According to Leeder and Watt [29] primary sorption sites on protein molecules have different hydrophilicity, so that the highest number of H_2O moles per mole specific site was calculated for $-NH_2$ and –COOH groups (≈ 1 at 20% RH and ≈ 2.5 at 80% RH), a medium value was found for the phenyl–OH groups (between 0.2–1.75), and the lowest hydrophilicity was exhibited by aliphatic–OH, –CONH–, $-CONH_2$ and –NH– groups (between 0.1–0.5). Therefore, the water sorption power of grains depends not only on protein content but also on protein composition.

TABLE III Hydration capacity (*a*) obtained from water imbibition by individual grains and content of proteins and soluble carbohydrates in different cereals and legumes

Variety	Number of grains	Hydration capacity (*a*) (g/g)	Proteins[a] (%)	Soluble carbohydrates[a] (%)
Wheat	6	0.44 ± 0.04	10.5	78.6
Triticale	7	0.50 ± 0.03	15.0	78.7
Maize[b]	–	0.48	10.0	80.0
Horsebean	4	1.10 ± 0.04	29.0	60.3
Mango beans	7	1.48 ± 0.05	26.0	50.0
Giant beans	8	1.48 ± 0.09	24.1	66.3
Pea	16	1.23 ± 0.06	27.5	62.4
Cowpea	4	1.48 ± 0.14	24.4	62.3
Chickpea	16	1.42 ± 0.08	20.3	58.5
White lupin	7	1.82 ± 0.05	33.7	39.0
Lentil	4	1.21 ± 0.02	28.5	52.0

[a] These data are average values taken from Kent [25], Arora [26], and Bekrić [27].
[b] Average value ($a = 0.48 \pm 0.15$ g water/g dry matter) for 30 maize genotypes [18].

Rupley *et al.* [30] have divided water in interaction with globular proteins into four hydration levels. The first corresponds with < 0.07 g water/g protein where the water molecules are in strong interaction with charged groups. The second level is between 0.07 and 0.25 g water/g protein when clusters of water molecules form and continue to grow until most of the protein surface is covered. The next hydration level between 0.25 and 0.38 g water/g protein was assigned to condensation of water, and finally at water contents above 0.38 g water/g protein we find conditions of full hydration. According to Saenger [31] the surface of 1 g of a protein can tightly bind about 0.25–0.75 g water. It can be seen that our value for water content at $T_{1\,\mathrm{min}}$ corresponded with the second and the third hydration levels in the above notation.

We also note a strong correspondence between our results and a recent study by Bruni and Carl Leopold [32] on adsorption isotherms and protonic conductivity (dielectric measurement) of corn embryo and endosperm as a function of hydration level. These authors found the peak of differential enthalpy (ΔH) for corn embryo at 0.08 g water/g dry matter, and an explosive growth of protonic conductivity above 0.082 g water/g dry embryo. Therefore they assigned this water to the water monolayer, in excellent agreement with our extrapolated value from Fig. 2. They also showed that completion of the primary hydration process in corn embryo (main constituents are proteins and lipids) is achieved at 0.24 g/g, while in endosperm (main constituent, starch) it is reached at 0.17 g/g. When the seed is taken into account the authors called the region between 0.08 and 0.25 g water/g dry matter a region of restricted or "local" metabolism. It is believed that the corn embryo at water contents below 0.12 g/g at room temperature exist in a glassy state, i.e. in the state of a liquid solution with the viscosity of a solid [33].

3.2. Water binding to polymers in grain powders from TSDC measurements

In Fig. 3 we show TSDC thermograms measured on a sample of cowpea at three different water contents. The thermograms exhibit two dispersion regions: a low temperature dispersion with maxima in depolarization current (peaks) between 120 and 200 K and a high-temperature dispersion with peaks between 200 and 300 K. Both dispersions shift to lower temperatures with increasing water content (i.e. the corresponding relaxation mechanisms become faster) and increase in magnitude. The thermograms shown in Fig. 3 are typical for the four kinds of seeds measured by TSDC in this work.

It has been discussed in a previous work [13] based on several TSDC studies in hydrated proteins and saccharides [34, 35], as well as in other hydrated materials, how information on the hydration in material under investigation can be extracted from TSDC measurements. The main points of that discussion in relation to our results in seeds are as follows. A significant contribution to the low-temperature dispersion at high water contents arises from the reorientation of

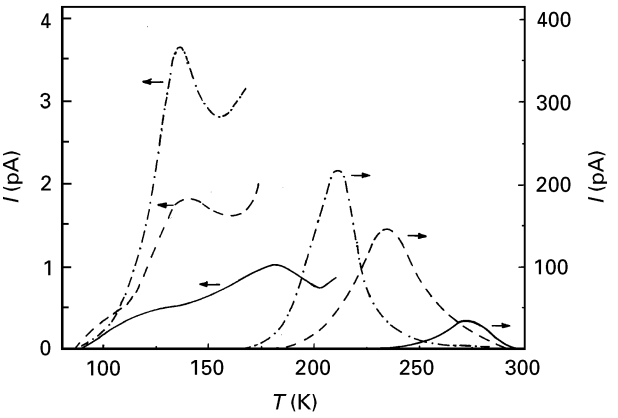

Figure 3 TSDC thermograms measured on cowpea seeds at threee different water contents (g water/g dry matter). Key: *h*: 0.07 (——), 0.16 (– – –) and 0.22 (–·–).

water molecules in frozen water clusters or water layers around the primary hydration sites. Water molecules bound at primary hydration sites are tightly (irrotationally) bound, i.e. they do not contribute by reorientation to the TSDC thermograms. Bulk (free) water exhibits a TSDC peak at about 120 K similar to macroscopic polycrystalline pure ice [13]. At water contents lower than the critical value for the completion of the primary hydration sites, the low-temperature dispersion is dominated by the relaxation of small polar groups, such as side chains in proteins and saccharides [13, 34, 35], often plasticized by water. Several relaxations may contribute to the high-temperature dispersion: relaxation of larger polar parts of macromolecules, conductivity relaxations due to transport of ions, very often protons, and space charge relaxations [13, 34, 35]. In some systems the high-temperature dispersion has been related to freezing-in of molecular mobility, similar to a glass transition [13, 36].

In what follows we focus our attention on the properties of and the informations extracted from the low-temperature dispersion. The properties of the high-temperature dispersion, related to a glass or glass-like transition in seeds, a topic of significant fundamental and practical interest [37], will be discussed elsewhere.

In Fig. 4 we show the dependence of the temperature of current maximum (peak temperature) T_m and of the normalized current maximum I_n on water content h for the low temperature dispersion measured on chickpea seeds. I_n is defined as the current maximum divided by heating rate and polarizing field and is a measure of the number of relaxing units contributing to the peak [13]. T_m in Fig. 4 decreases continually with h for $h < 0.15$–0.20 and then becomes constant, $T_m = 130$–135 K. I_n increases slowly with h for $h < 0.15$–0.20 whereas the increase with h is significant for larger h. Similar results were obtained with beans and cowpea seeds. They can be interpreted as follows. For water contents with h smaller than 0.15–0.20, water molecules are tightly (irrotationally) bound at primary hydration sites and do not contribute by their reorientation to the low-temperature dispersion. The changes observed in Fig. 4 for $h < 0.15$–0.20 are due to

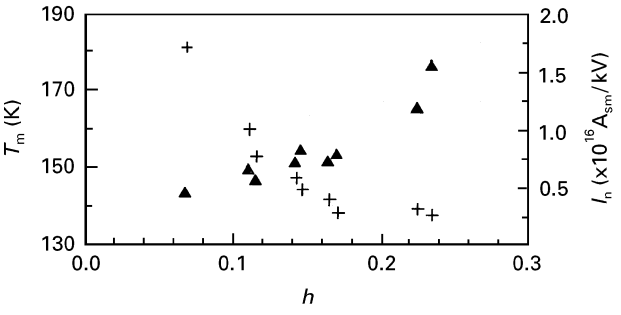

Figure 4 Peak temperature T_m(+) and normalized current maximum I_n(▲) at the low-temperature TSDC dispersion measured on chickpea seeds (legume) versus water content h.

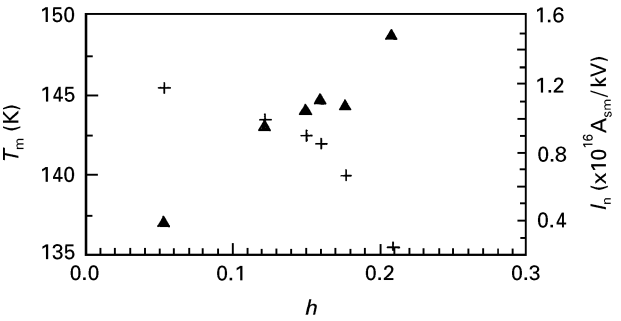

Figure 5 Peak temperature T_m(+) and normalized current maximum I_n(▲) at the low-temperature TSDC dispersion measured on wheat seeds (cereal) versus water content h.

the plasticizing action of water on side chain relaxations [34, 35]. For $h > 0.15$–0.20, water molecules in clusters around the primary hydration sites contribute by their reorientation to the low-temperature dispersion, which is now dominated by a peak at about 130–135 K, a temperature region characteristic for loosely bound water molecules [13]. The result of that is that T_m becomes approximately constant, $T_m =$ 130–135 K, whereas I_n increases significantly with h. (We expect a linear increase of I_n with h [13]; measurements at higher water contents are needed to verify this prediction.) Considering together the results obtained with the three kinds of seeds showing similar behaviour, i.e. beans, chickpea and cowpea, the amount of tightly bound water in these seeds is determined as 0.18 ± 0.02 g water/g dry matter.

The results obtained with wheat, the only one cereal measured by TSDC, are less conclusive (Fig. 5). They can be interpreted in terms of the amount of tightly bound water at primary hydration sites being about 0.10 g water/g dry matter. The decrease of T_1 with h at larger values of h in Fig. 5 probably reflects the formation of larger water clusters around the primary hydration sites (or hydration in multilayers), a behaviour typical for may proteins and saccharides [13, 34, 35]. However, we think that further work is needed to verify these results and to investigate whether the behaviour of wheat is typical for cereals, as suggested by the NMR results. Experiments along these lines are now in preparation. They will also provide a basis for critically comparing with each other the absolute values for the amount of bound water obtained by NMR and by dielectric spectrosocpy. We would like to stress that these absolute values may be very different, since the techniques are different and the definition of the terms bound and free depends on the experimental method used [38].

3.3. Water imbibition of grains

Water imbibition of grains can be used to evaluate their maximum hydration capacity, a (Equation 1). The imbibition involves two simultaneous processess: entry of water into the kernel and swelling of polymers, i.e. proteins, starch [39].

The imbibition equation was applied to individual grains and the resulting hydration capacity for 11 cereals and legumes were collected in Table III. In Fig. 6 we show for the sake of illustration the imbibition curves for individual grains of wheat, cowpea and lupin, showing different rates of water entry as well as different hydration capacities due to swelling of their proteins (10.5, 24.4 and 33.7%, respectively, see Table III). The hydration capacity (a) is in high correlation with protein content for the 11 species investigated ($r = 0.83$). The ratio between hydration capacity and water content at $T_{1\min}$ (primary hydration shell) was named the swelling index and it is also highly correlated with protein content (Fig. 7, $r = 0.84$).

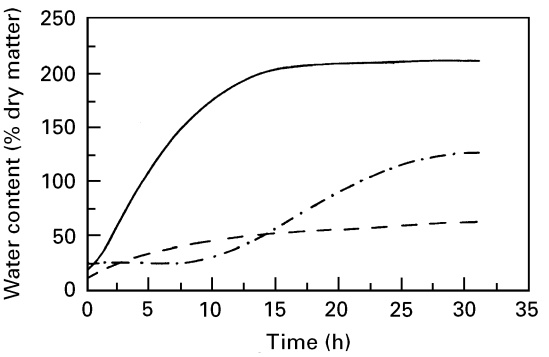

Figure 6 Typical imbibition curves for single grains of wheat, cowpea and white lupin. The experimental points were excluded for the sake of clarity, since all points lay on the drawn curves.

3.4. Common model of hydration from NMR and TSDC measurements

It was shown in Section 3.1 that $T_{1\,\min}$ is shifted to higher hydration levels when protein content in the seed increases. But, if we now plot T_1 as a function of per cent of maximum hydration (estimated by imbibition) for wheat (cereal) and horsebean (legume), the resulting points for these two types almost overlap, and moreover they practically follow a similar curve for a quite different biological object *Artemia* cyst (Fig. 8). The corresponding curve for *Artemia* cysts and the linear dependence between T_1 and the per cent of maximum hydration with obvious absence of $T_{1\,\min}$ obtained for glycerinated muscle was taken from Clegg [8]. It seems that in such biological materials containing proteins, starch and other biopolymers $T_{1\,\min}$ appears at 25–30% of maximum hydration.

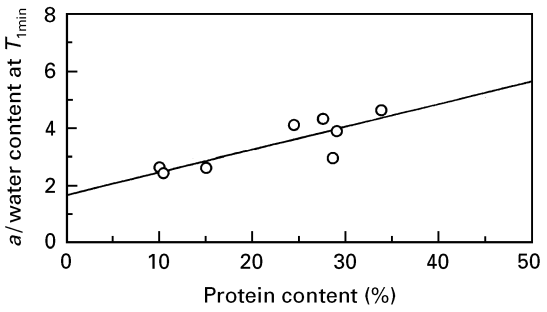

Figure 7 Swelling index (ratio of hydration capacity and primary hydration sphere) versus protein content for 11 cereals and legumes ($r = 0.84$) .

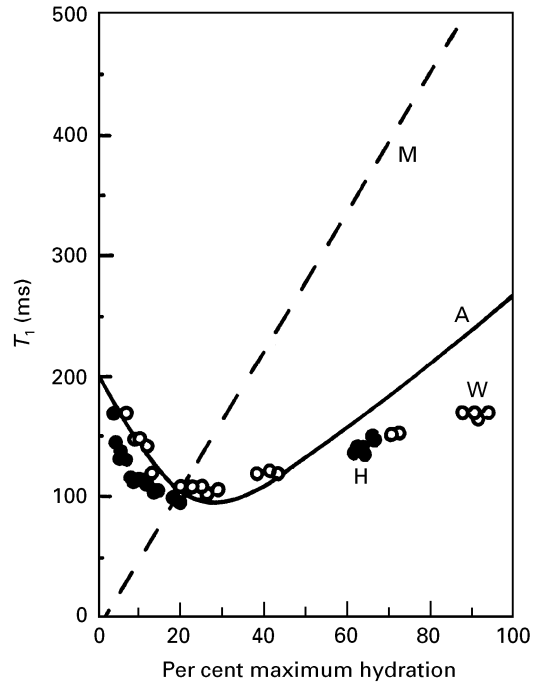

Figure 8 T_1 plotted against per cent of maximum hydration for a cereal (wheat, W) and a legume (horse bean, H). The solid curve A was taken from Clegg [8] for *Artemia* cysts, while the dotted line M shows the literature data from glycerinated muscle taken from the same reference.

This is actually the primary hydration sphere where water molecules can form larger clusters around primary hydration sites on biopolymers or they can hydrate in multilayers; it is presumed that water molecules in this sphere have restricted mobility (10^{-9}–10^{-8} s) compared with bulk water (10^{-12}–10^{-11} s), and here they can exchange their proton spins with the corresponding proton spins on the biopolymer surface, as has been discussed earlier.

This picture obtained with NMR is in qualitative correspondence with that emerging from TSDC measurements, the only difference being a somewhat lower value for the primary hydration sphere found by the dielectric measurements.

4. Conclusions

These combined proton NMR relaxation, TSDC and imbibition studies of hydration processes in cereal and legume grains revealed the following.

1. All proton NMR relaxation measurements on intact grains showed V-type curves for T_1 versus hydration as a result of spin exchange between water protons and protons on the biopolymers surface (proteins, starch) which is dominant at low water contents.

2. There is a tendency for the position of $T_{1\,\min}$ to shift to higher water contents when protein content of the grains increases, ranging from 0.18 g/g for wheat (cereal) to 0.41 g/g for lentil (legume). The observed correlation between these two parameters was very high ($r = 0.90$), and extrapolation of the straight line for zero protein content gave water content of 0.08 g/g which we have assigned to a water monolayer around starch (irrotationally bound) in agreement with literature data.

3. TSDC measurements on wheat, which was taken as a representative of cereals, suggested that tightly bound water at primary hydration sites is around 0.10 g water/g dry matter, close to the value for a water monolayer on starch obtained with NMR. On the other hand tightly bound water of 0.18 g/g in three legumes is also in qualitative agreement with conclusions drawn from the NMR measurements.

4. Maximum hydration capacity (a) of intact kernels obtained by water imbibition studies also showed a linear correlation with protein content. Swelling index (a/water content at $T_{1\,\min}$) was in high positive correlation with protein content ($r = 0.83$).

5. Replotting T_1 versus per cent of maximum hydration gave similar position of $T_{1\,\min}$ for cereals and legumes at 25–30%. This could be associated with a primary hydration spheres around biopolymers. Further increase in hydration results in the swelling of biopolymers until maximum hydration capacity was attained.

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