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Quantitative determination of bound water in wheat starch by time domain NMR spectroscopy

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Abstract

Above a water content of about 0.42 g per g of dry starch (DS), the Carr–Purcell–Meiboom–Gill (CPMG) relaxation-curve analysis of wheat starch–water suspensions shows two components. The intensity of the short component ($T_2 \approx 1.5$ ms) corresponding to 'bound' water increases over the full hydration range studied from 0.55 to 0.9 g of water per g of DS. Assuming an intermediate exchange rate between 'weakly bound' and 'bound' water at 20 °C, a constant 'bound' water content of 0.170 ± 0.008 g of water per g of DS has been obtained. The existence of a two-site exchange process has been argued to validate this amount of 'bound' water. This result takes into account the fact that about 7% of the starch gives a 'liquid-like' signal. A complete treatment of the FID signal (solid and liquid parts) allowed us to determine the signal in arbitrary units per atom g of hydrogen present in the samples. Thus, we have been able to transpose the signal obtained by a relaxation curves analysis (in arbitrary units), to grams of water. This 'bound' water, determined through NMR CPMG sequence, would correspond to a bilayer of water. The concept of 'potentially free' water has been introduced to explain the lack of noticeable 'weakly bound' water by a CPMG sequence in the range of 0.17–0.42 g water content. The NMR data are discussed in relation to similar measurements made on sorption curves and in relation to plasticization. Overall, the concept of several populations of water molecules presenting different mobilities is reinforced.

Keywords: Wheat starch; Bound water; NMR

1. Introduction

The quantity of water bound to the macromolecules or other ingredients in a foodstuff is an important parameter since it determines, amongst other things, the microbial growth potential and its optimal drying to allow a rehydration in a reversible manner [1]. One of the methods to determine the amount of 'bound' water consists of a bi-exponential analysis of the CPMG [2] relaxation curves

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obtained with high and low field NMR spectroscopy [3–5] on products containing free water. The analysis gives a short component of the order of milliseconds attributed to the 'bound' water, and a long component whose value increases with the water content. This method has been used to study milk powder [4], crackers [6], proteins [7,8], sugars [9] and starch [10,11], as well as biological systems [12], tissues [13] and the denaturation of proteins [14].

In a water–starch suspension, three kinds of protons can be found: water protons, starch non-exchangeable protons (C–H and some hydroxyl functions of the crystalline phase) and exchangeable protons. Up to now, the quantity of 'bound' water has been determined by considering that the relaxation curve signal corresponds to the full amount of water in the sample and only to this water. However, the signal measured $70\,\mu$s after the pulse, usually representing the liquid-phase signal, evolves with a slope which depends on the water content [15]. This clearly shows that different situations can be encountered: (i) The water fully or partly participates in the solid phase signal; (ii) on the contrary, a part of the macromolecular exchangeable hydrogen atoms participates in the liquid phase signal; and (iii) a part of the macromolecules has sufficient mobility to give a 'liquid' signal. We can see that, in these conditions, the simple attribution of the liquid-phase signal to the totality of the aqueous phase may be incorrect. This work aims to quantify the 'liquid' protonic populations in water–starch suspensions.

2. Experimental

Samples.—Wheat starch was commercial grade from CERESTAR (Neuilly sur Seine, France). Moisture content was 12.5%, as determined by a vacuum oven (AOAC, 1980) using 60 °C under 10^2 Pa for 24 h. All other water contents given are in g of water per g of dry starch (g W/g DS).

NMR measurements.—A Bruker PC 120 Minispec, with a 0.47 T magnetic field operating at a resonance frequency of 20 MHz has been used for measurements. The probehead (10 mm) was kept at 20 ± 1 °C and the temperature controlled by circulating fluorocarbon (FC40 fluorinert from 3M). For the free induction decay (FID) signal (signal after a 90° pulse), the acquisition parameters used were: relaxation delay, 2 s; 90° pulse width between 1.4 and 1.6 ms; attenuation, 23 db. The FID signal has been sampled with a Team490 acquisition card from Bakker Electronics as follows: data points, 1024; number of acquisitions, 128; time intervals, 1 and $5\,\mu$s.

Proton transverse relaxation times T_2 have been measured using the CPMG spin-echo sequence: $90^\circ x\text{-}(\tau\text{-}180^\circ y\text{-}2\tau\text{–}180^\circ y\text{-}\tau\text{–Acq})n'$. The pulse spacing time 2τ had a value of 0.2 ms. The number of echoes has been chosen between 40 and 300 in order to register the complete decay of the signal. The small memory of the spectrometer required that, when n' was greater than 169, every second and fourth echo was recorded one after the other, in order to sample the beginning, and then the end of the relaxation curve decay. The curves were then merged. All the intensities are expressed in arbitrary units (a.u.). The echo decay has been fitted both to a bi-exponential and/or to a mono-exponential decay (Bruker program EXP-SUP). Different results were obtained with the CPMG sequence when recorded in the PSD or the diode mode; the diode mode yielded a more stable signal than the PSD mode, and was then thought to be better adapted when measurements obtained over several days were to be compared, although measurements were not perfectly quantitative over the 0–200 a.u. range. The first measurement series (Table 1) was therefore made using the diode mode. On the contrary, the further series measurements (Table 2), each made within one day, were made through the

Table 1
NMR relaxation parameters obtained for wheat starch according to the water content, through CPMG (diode mode detection) relaxation curves

	W (g H_2O/g DS)	T_{2a} (ms)	T_{2b} (ms)	P_a (%)	I_a (a.u.)	I_0 (a.u.)
S1	0.143	0.38	—	100	225	225
S2	0.260	1.26	—	100	510	510
S3	0.380	1.76	—	100	820	820
S4	0.467	1.89	3.32	55.4	618	1115
S5	0.476	1.37	3.23	33.0	376	1140
S6	0.534	1.34	3.59	29.0	365	1260
S7	0.548	1.41	3.98	29.4	385	1310
S8	0.594	1.35	5.02	33.3	471	1415
S9	0.619	1.52	5.54	37.5	536	1430
S10	0.661	1.49	7.01	36.5	573	1570
S11	0.676	1.59	7.02	36.7	574	1565
S12	0.728	1.50	8.72	35.6	612	1720
S13	0.780	1.58	9.07	35.0	625	1785
S14	0.836	1.63	11.1	34.6	673	1945
S15	0.837	1.58	10.9	34.0	656	1930
S16	0.858	1.64	11.2	34.3	677	1975
S17	0.869	1.71	11.7	32.8	646	1970
S18	0.948	1.71	13.4	31.7	685	2160

W, water content per g of dry starch.

Table 2
NMR relaxation parameters obtained for wheat starch according to the water content in ranges 0.2–0.3 and 0.4–0.47 g per g of dry starch. The phase sensitive detection mode has been used for these measurements

W (g H_2O/g DS)	T_{2a} (ms)	T_{2b} (ms)	I_a (a.u.)	I_b (a.u.)
0.200	1.5	9.4	827	16
0.235	1.7	8.2	974	21
0.250	1.8	12.3	1010	11
0.272	1.8	8.9	1170	18
0.305	1.9	31.0	1160	10
0.397	2.1	7.7	1560	50
0.427	2.2	5.3	1480	241
0.447	2.2	5.6	1500	271
0.460	2.3	6.0	1410	313
0.474	2.3	5.6	1460	459

PSD mode to obtain more accurate results than with the diode mode. There is, in fact, a slight shift between the T_2 values obtained by the two detection modes. The diode mode has given smaller values than the PSD mode but the appearance threshold of observable ‘weakly bound’ water was the same for the two measurement methods.

3. Results and discussion

^{17}O and ^{2}H NMR studies have many advantages over ^{1}H studies especially as their relaxation mechanism is dominated by the quadrupolar relaxation which avoids cross-relaxation phenomena, and ^{17}O chemical exchange mechanisms are slow enough within the time scale of the NMR measurement to be considered as negligible [16–19]. However, the sensitivity of ^{17}O, 2.9×10^{-2} compared to 1 for ^{1}H at the same concentration, does not allow measurements on samples with a low $H_2{}^{17}O$ content. Previous reports on water–starch suspensions have therefore been made on samples with water contents greater than 1 g W/g DS. At this water content, wheat starch suspensions are not stable and starch granules deposit during the NMR measurements. Yakubu et al. studying potato starch by ^{17}O NMR spectroscopy, found only one state of anisotropically ‘bound’ water at 1.38 g W/g DS [3,20,21]. However, depending on its origin, starch does not show the same behaviour towards water. For example, wheat starch suspensions are not stable at this water content and starch granules deposit during the NMR spectroscopy experiment. The use of ^{1}H T_2 relaxation measurements permits the analysis of the state of water at lower water contents and provide evidence that in wheat starch the anisotropically ‘bound’ water exists in more than one state.

The mono- or bi-exponential analysis by the NLREG program (discrete method) [23] of the CPMG relaxation curves obtained on water–starch suspensions ranging from 0.143 to 0.948 g W/g DS gave the T_2 relaxation times and the populations shown in Table 1. We note that the short T_2 is of the order of size of the T_2 measured for water bound to corn starch ($T_2 = 1.43$ ms) [22] or in milk powder [4].

The fitting of these CPMG relaxation curves by a continuous method (90 data points) (CONTIN, [24,25]) gave two distinct components from a water content of 0.548 g W/g DS (Fig. 1). It can be seen that the two methods gave identical results except for the S_4–S_6 samples which correspond to the beginning of the separation of the two components (short and long). Indeed, by simulations, we checked that with a large distribution of relaxation times, discrete methods tend to give two components on both sides of the maximum of distribution.

The short component T_2 slightly increases from 1.3 to 1.7 ms as the water content increases. From a water content of 0.548 g W/g DS, the quantity of ‘bound’ water regularly inflates over the full range studied even when a large amount of ‘weakly bound’ water is present, which is surprising.

‘Weakly bound’ water–‘bound’ water exchange.— However, the occurrence of a ‘weakly bound’ water–‘bound’ water exchange with an intermediate rate constant between a slow and a fast exchange can be expected [5]. In the case of a fast exchange, only one component is expected to be obtained.

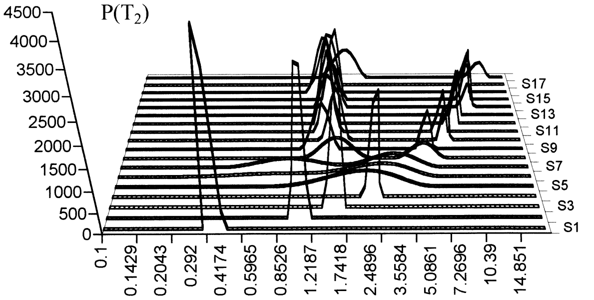

Fig. 1. T_2 relaxation times determined by the Contin program from CPMG relaxation curves according to the water content. The water content of different samples, *Si*, are given Table 1.

In order to check the occurrence of an exchange phenomenon between 'weakly bound' and 'bound' water, T_2 measurements were made at different temperatures, 0, 20 and 55 °C on a starch sample with 0.61 g W/g DS. We can see from Fig. 2 that the difference between the T_2 of the peak of 'weakly bound' water and that of 'bound' water decreases as the temperature increases: 8.9, 6 and 2.9 ms respectively. This indicates an exchange phenomenon. The effect of an increase in the exchange rate is to average the two T_2 relaxation times to give a single peak.

The T_2 of the 'weakly bound' water peak decreases from 11 ms ($T=0$ °C) to 4.4 ms ($T=55$ °C), although the single effect of temperature without an exchange phenomenon would have been to increase it; the behaviour of the 'weakly bound' water peak is thus compatible with an exchange phenomenon. However, the T_2 of the 'bound' water peak decreases from 2.2 to 1.5 ms instead of increasing. However, this shift of -0.7 ms can only be due to measurement errors because of the inaccuracy of the first data points of CPMG relaxation curves [26].

By high resolution NMR spectroscopy, using the WISE sequence with a short mixing time, Kulik et al. [27] estimated that in native corn starch, in A and B forms, 28% of water is bound to the macromolecules, giving a 'bound' water quantity of 0.038 g W/g DS. This water should be considered as 'strongly bound' water rather than as 'bound' water within the general meaning of this term, that is to say corresponding to a relaxation time of the order of 1 ms.

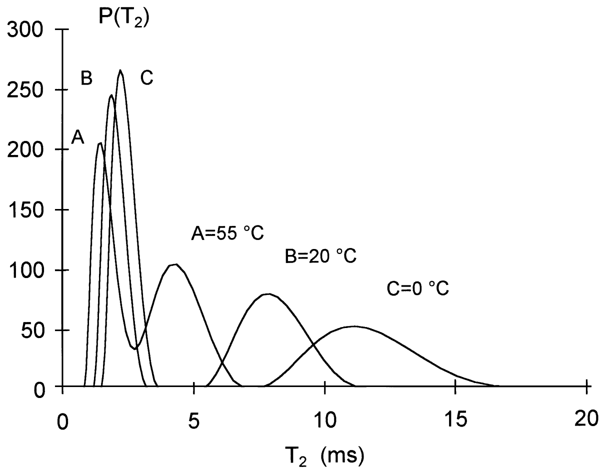

Fig. 2. Evolution of the T_2 distribution of wheat starch (0.6 g W/g DS), according to the temperature: (A) 55 °C, (B) 20 °C and (C) 0 °C.

Different experiments made on 0.20, 0.235, and 0.25 g water content samples according to temperature showed that a 'strongly bound'–'bound' water exchange is not apparent.

A two-site exchange mechanism can therefore be considered as adequate for an analysis of the T_2 relaxation times of 'bound' and 'weakly bound' water.

The NMR parameters determined from the relaxation curves must no longer be considered as true values but as apparent values [28]. The two sets of parameters are linked by the relation (subscript 'a' for apparent, 'b' for bound, 'f' for free; a_b and a_f are the apparent populations):

$$\sum \frac{P_i}{T_{2i}} = \frac{a_b}{T_{2ab}} + \frac{a_f}{T_{2af}} \tag{1}$$

which simply translates the fact that the weighted average relaxation rate is identical in both cases.

It has previously been shown that in starch suspensions, except for glucosyl residues ($\approx 7.5\%$) which have a 'liquid-like' behaviour [15], the hydrogen atoms of the starch hydroxyl groups contribute to the 'solid signal'. In this case, we can consider the presence of a two-site exchange and the following equation can be written:

$$\frac{P_b}{T_{2b}} + \frac{P_f}{T_{2f}} = \frac{a_b}{T_{2ab}} + \frac{a_f}{T_{2af}} \tag{2}$$

and with $P_b + P_f = 1$ and $a_f + a_b = 1$, we obtain:

$$P_b = \frac{\left(\frac{a_b}{T_{2ab}} + \frac{a_f}{T_{2af}} - \frac{1}{T_{2f}}\right)}{\left(\frac{1}{T_{2b}} - \frac{1}{T_{2f}}\right)} \tag{3}$$

Assuming that the relaxation time of 'bound' water, of the order of 1 ms, is much smaller than the relaxation time of free water (2.2 s at 20 MHz and at 20 °C for distilled water), we can assume that, even with a small amount of matter in solution, the relaxation time of the most mobile phase of water, T_{2f}, will be much longer than for 'bound' water. Therefore:

$$\frac{1}{T_{2f}} < \frac{1}{T_{2b}}$$

Moreover, in the hydration range studied (from 0.13 to 0.94 g W/g DS), the 'bound' water concentration can never be considered as insignificant compared with the 'free' water concentration. In this case, eq (3) is as follows:

$$P_b = T_{2b}\left(\frac{a_b}{T_{2ab}} + \frac{a_f}{T_{2af}}\right) \quad (4)$$

The problem now is to determine the true value of the 'bound' water relaxation time. It seems, however, difficult to obtain this value from a sample which only contains this type of water (i.e. native starch), because of the low signal amplitude and of the rapid decrease of the relaxation curve. Indeed, it can be, a priori, considered that native starch, for example, does not contain free water since it can be kept for several months at room temperature.

The evolution of the short T_2 (T_{2ab}) and of the long T_2 (T_{2af}) has been plotted in Fig. 3 according to the water content (W). We note that above 0.5 g W/g DS, these two parameters have a linear evolution:

$$T_{2ab} = 0.94 + 0.84W;\ r^2 = 0.838;\ n = 13 \quad (5)$$

$$T_{2af} = -9.0 + 24W;\ r^2 = 0.995;\ n = 13 \quad (6)$$

The intersection of these two straight lines has the following coordinates: $W = 0.434$ g of water and $T_{2a} = 1.3$ ms. This point corresponds to the appearance threshold (apparent) of the 'weakly bound' water.

If we take a T_{2b} value of 0.94 ms, corresponding to the 'bound' water T_{2ab} for a null water content (eq (5) with $W = 0$), we obtain $I_b = 494 \pm 15$ a.u./g DS, corresponding to 0.215 g W/g DS.

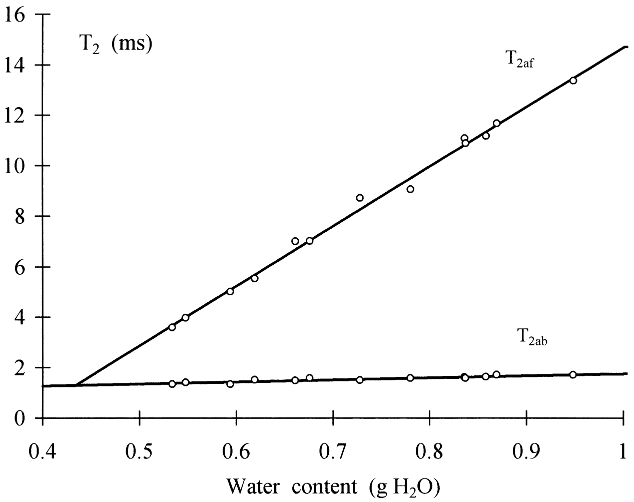

Fig. 3. Evolution of 'short' and 'long' T_2 relaxation times of wheat starch according to the water content (per g of dry matter).

With a starch-water sample (0.833 g W/g DS, 116.8×10^{-3} At g of hydrogen), a total signal (I_0) of 2380 a.u. corresponding to a 20 385 a.u./At g H signal, was obtained. We previously showed that the determination method of such a value was repeatable [29].

However, due to imperfections in the 180° pulse [26], for any liquid sample the ratio of the total signal I_0 CPMG/I_0 FID is 1.015. The signal for each atom g of hydrogen measured through a CPMG sequence therefore becomes 20690 a.u.

The 494 ±15 a.u./g DS signal therefore corresponds to 23.9×10^{-3} At g of hydrogen, that is to say 0.215 ± 0.008 g W/g DS.

We must also consider that the starch chain ends may have a great enough mobility to be seen through a CPMG sequence. To check this assumption, a ^{2}H exchanged starch suspension was been prepared. The exchange process was performed eight times by adding about 1 g of deuterium oxide to 1 g of wet starch (about 10% of water content). After five exchanges, a constant NMR signal was obtained. The total signal of the CPMG relaxation curve was of 75 a.u. under the same experimental conditions as those used for the first experiment series. Taking into account that the 'OH' functions of these starch residues are totally ^{2}H exchanged, this signal corresponds to 107 a.u. in a ^{1}H medium, that is to say to 45 mg of equivalent water. This value must be subtracted from the 0.215 g of water previously obtained, which gives 0.17 g of 'bound' water per g of DS.

Threshold of appearance of 'weakly bound' water.—To confirm this threshold of 0.17 g W/g DS, we studied five samples whose water content was slightly greater than this value, in the 0.20–0.30 g W/g DS range. The T_2 value of the 'short' component of these five samples slightly increased with the water content (Table 2), from 1.5 to 1.9 ms as well as its intensity. On the other hand, whatever the water content, a 'long' component was found at about $T_2 = 10$ ms, but its intensity remained practically constant, of the order of 15 a.u.; it can then be taken as an artefact and was not taken into account.

The spin–spin relaxation times (T_2) method does not detect 'weakly bound' water up to 0.305 g W/ g DS, although the theoretical appearance threshold of 'weakly bound' water (0.17 g W/g DS) is exceeded. The reasons for this discrepancy are discussed below.

'Bound' water is in chemical interaction with macromolecules. For water contents slightly higher than the appearance threshold of 'weakly bound' water, the additional water forms a new layer. The water of this layer has a low degree of freedom because it cannot rapidly exchange with the molecules of the 'bound' water layers. This water has then a T_2 relaxation time slightly higher than that of 'bound' water. However, the little mobility of this water is due only to physical reasons. Despite its small T_2 relaxation time, we can speak of 'potentially free' water. In fact, when the water content increases, these water molecules can exchange with their new neighbouring water molecules and therefore gain a greater mobility. Their T_2 increases and differs from the T_2 of 'bound' water. At this stage, this 'weakly bound' water can be detected by the CONTIN program from CPMG relaxation curves. We can see (Table 2) that the appearance threshold of a significant and long component is of about 0.42 g W/g DS. This water content value is in good agreement with those determined by the intersection of the two straight lines (eqs (5) and (6)).

'Bound' water content in the amorphous phase.— Interactions of starch granules with water cannot be reduced to a simple adsorption, as local structures inside the granule favour the intimate organisation of solvent molecules with the polymer chains in a polymer–solvent complex or crystallosolvate.

At the macromolecular level, starch granules present an organisation leading to a spherulite-like structure, with a radial cohesiveness due to the chain axis of the macromolecules and a tangential one induced by intermolecular bonds. These are responsible for the formation of crystalline regions by aggregation of a great number of chains. These crystallites are separated by amorphous regions. Inside a native starch granule, water molecules are distributed between crystalline and amorphous regions. The 'fringed micelle' model is often used to illustrate such a tridimensional network composed of amorphous areas crosslinked by microcrystallites [30]. Currently accepted values for absolute crystallinity vary from 20 to 39% DS according to the technique used: solid-state NMR, X-ray diffractometry, density, acid hydrolysis [31]. With the A-type model proposed by Buleon and his co-workers [32], four water molecules are trapped inside each unit cell, where 12 glucopyranosyl units are located. Therefore, water molecules represent only 72 g for 1944 g DS, or 0.037 g W/g DS crystallites. Thus, a measurement of 0.170 g of 'bound water' per g DS for total native wheat starch granules indicates that there is between 0.20 and 0.26 g of 'bound' water per g DS for the chain segments located in the amorphous zones.

These values may be compared with the results deduced from moisture sorption isotherms, which is considered the 'classical' way to describe biopolymer–water relationships with solid state substrates. Interpreting these sorption curves according to the Brunauer–Emmett–Teller (BET) [33] equation is based upon experimental data, gained on a broad range of a_w values from 0 to 0.90. This equation enables us to determine w_m, the amount of water adsorbed when the surface of the adsorbent sample (i.e. starch) is covered with a single layer of molecules. This fraction corresponds to sorption water determined in the region of water activity, between 0 and 0.2, where the BET equation is most precise. Bizot [34] found 0.092 g W/g DS of 'bound' water at 25 °C. Thus, it appears that the 'bound' water measured by a NMR CPMG sequence is greater than the monolayer of water proposed by the BET model. The 'bound' water, determined by NMR, represents further layering of water molecules, but these must be presumed not to be in direct contact with the carbohydrate. An aqueous multi-layer can be proposed while capillary effects can also be invoked. However, such a level of hydration remains far below the upper limit of water absorption, quoted by French [30] to be around 50% on a dry basis for native starch granules.

A further way to interpret these data is related to the concept of plasticization. This mobility change is related either to the presence of small solute molecules (water) or to a temperature increase. Both factors promote chain mobility over a long range (6–10 glucosyl units): material properties change from brittle (vitreous) to rubbery behaviour. Several studies have demonstrated that, at a low moisture content, water molecules induce an antiplasticization behaviour, i.e. a decrease of yield at break and an increase of maximum stress [35]. The general trend is a strong decline of T_g with water content. Potato starch presents a pure polymer T_g of 589 K in an anhydrous state, the value of which shifts to 298 K at 0.282 g W/g DS [34]. In contrast, when the fine structure of α-glucans changes from linear (amylose) to highly branched (phytoglycogen), the moisture content required to a T_g at room temperature decreases from 0.21 to 0.19 g W/g DM: the plasticizer concentration has a stronger influence than the fine structure of α-glucan for the location of plasticization at room temperature. Below T_g, water molecules are supposed to be highly 'linked' to α-glucan chains, producing a cross linking effect at low concentrations. In measurements made by NMR spectroscopy at 25 °C, we observe a value for the threshold above which more mobile water molecules are present of 0.20 g W/g DS, in close agreement with data obtained by other methods. This critical water level, detected by ^{1}H mobility, induces a change in α-glucan mobility, indicating a coupling between the mobility of water and α-glucan segments. This behaviour has been suggested to explain antiplasticization in starch–glycerol films [36]. The same tendency has been noticed on Young's modulus for ice-cream wafers, where the presence of water led to a classical plasticization. Attenburrow and Davies [37], using NMR spectroscopy, noticed that the signal characteristic of 'freezable' water at -10 °C is present only at moisture content of 28% or more. NMR spectroscopy appears to be a potentially powerful technique for studying the effects of plasticization, in contrast to most spectroscopy which focus on polymer or material properties.

4. Conclusion

The occurrence of a 'bound' water–'weakly bound' water exchange phenomenon at an intermediate rate has been clearly demonstrated to occur in wheat starch suspensions. In contrast, the presence of a 'tightly bound' water–'bound' water exchange has not been detected, in agreement with results obtained with other nuclei. The apparent NMR parameters, obtained from the CPMG relaxation curves, have been corrected to take into account this two-site exchange mechanism. On the other hand, about 7% of the starch is found to give a liquid-like signal. These values have resulted in giving 0.17 g of 'bound' water per g of dry starch. If an average crystallinity of 30% is taken into account, a value of 0.23 g of 'bound' water per g of amorphous phase is obtained. As up to 0.42 g W/g DS NMR measurements have not shown the occurrence of 'weakly bound' water but only a single water population of 'bound' water, the notion of 'potential weakly bound' water has been introduced for the additional water between 0.17 and 0.42 g. The NMR data overall reinforce the concept of several populations of water molecules, presenting different mobilities, shown by similar measurements made on sorption curves and in relation to plasticization.

Acknowledgements

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