Quantitative analysis of wheat starch–water suspensions by pulsed NMR spectroscopy measurements

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Abstract

A rapid and non-destructive method, based on pulsed NMR spectroscopy, has been developed for the study of wheat starch–water interaction. It involves a Gaussian fitting of the FID signals from liquid and solid phases obtained by pulsed NMR spectroscopy, resulting in an exact and repeatable quantification of these different phases. Moreover, the standard addition method in the range of a water/starch ratio greater than 0.55 allows the use of water as an internal reference. This reference signal is in good agreement with the total amount of hydrogen atoms in the sample, regardless of the solid/liquid ratio. This allows, from wheat starch suspensions in deuterium oxide, a determination of the starch proportion which has a 'liquid' behaviour (7.5%) and of the quantity of exchangeable hydroxyl groups (2.6:3) in the wheat starch granule. © 1996 Elsevier Science Ltd.

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1. Introduction

Starch is the polymeric basic component of cereal foods when damp cooking methodologies are considered. Its molecular organization can be modified, either reversibly or irreversibly, under various conditions of which the most important factors are water content and temperature. The importance of starch–water interaction has been noticed by many authors using various methodologies. Differential scanning calorimetry

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(DSC), allows the measurement of temperature and enthalpy of starch changing state during a thermal treatment [1,2]. Small angle X-ray scattering [3] can afford to determine the crystalline structure of starch and the amount of water present in the amorphous phase which would affect the gelatinization. Sorption isotherm curves are interpreted as reflecting the evolution of the water molecules binding within the substrate. Three stages can be envisaged. The first, between water activity $(A_w) = 0$–0.2, i.e. water content between 0 and 0.1 g water per gram dry starch, corresponds to the adsorption of water molecules to primary sites of adsorption (polar groups) by hydrogen bonding (about 30.9 kJ/mol). Its mobility is almost zero and it cannot take part in either interaction or reaction. The second stage, between $A_w = 0.2$–0.7, i.e. 0.1–0.25 g water per gram dry starch, corresponds to the fixation of successive layers of water molecules, each new layer being superimposed on the former one, held in place by increasingly weak hydrogen bonding (28.4–23.4 kJ/mol). Fixation on polar sites, hidden in the first stages but now made accessible by the swelling, is also considered. The third stage ($A_w > 0.65$, i.e. water content > 0.25 g water per gram dry starch) corresponds to water, mobile enough to participate in reactions and to serve as a solvent, with the same characteristics as free water (activation energy $= 18.8$ kJ/mol). At saturation, starch granules can adsorb 0.4–0.54 g water per gram dry starch [4].

Nuclear magnetic resonance spectroscopy has been widely used to study water mobility in static conditions [5–7]. Richardson et al., using ^{2}H and ^{17}O NMR spectroscopy, have been able to determine different zones of water mobility in corn starch, depending in water content. In an initial study [5] covering a relatively low solid content, i.e. in a range of 10–70% starch corresponding to 0.43–9 g water per gram dry starch, three regions of decreasing water mobility were determined. Region I, from 10 to 40% starch, i.e. 1.5–9 g water per gram dry starch, was described by the isotropic two-state model with fast exchange. Region II, from 40 to 60% starch i.e. 0.66–1.5 g water per gram dry starch, was explained by a micro-aggregation of granules. Region III, from 60 to 70% starch i.e. 0.43–0.66 g water per gram dry starch, was explained on the basis of multilayered water formation. A later study [7], covering a concentration range from 71 to 96% starch i.e. 0.04–0.41 g water per gram dry starch, resulted in the determination of a fourth region. The limit of Region III is in fact 92% starch, i.e. 0.087 g water per gram dry starch, and Region IV, from 92 to 96% starch, corresponds to the monolayer. Kulik et al. [8], using the 2D ^{1}H–^{13}C heteronuclear wideline separation method (WISE) of solid state NMR, estimate that 28% of the water present in starch granules is bound to the polysaccharide, in both A and B types of starch. There is no preference for water to bind to a particular site of the glucose moiety, but the molecular mobility of water in type A is higher than in type B. This explains the lower gelatinization temperature of A-type starch. These studies were done by high resolution NMR, in solid or liquid state. However, some authors have also used low resolution NMR spectroscopy for the study of water–starch behaviour [9], through proton longitudinal (T_1) and transversal (T_2) relaxation times measurements, as well as by following their evolution during gelatinization [10]. This technique has also been used for the determination of water content [11] and to follow the retrogradation process in various conditions [12,13].

The purpose of this study is to quantify the physicochemical behaviour of starch and water protons when considering starch suspensions.

2. Experimental

Samples.—Wheat starch was commercial grade from CERESTAR (Haubourdin, France). Moisture content was 12.5%, as determined by drying in a vacuum oven at 60 °C under 10^2 Pa pressure for 24 h (AOAC, 1980). Deuterated wheat starch was prepared by dissolution in deuterium oxide (EURISO-TOP CEA, 99.9%) five times over 24 h periods and drying under mild conditions, at room temperature with phosphorpentoxid. Water content is expressed as gram water per gram dry matter and is noted as 'g H_2O/g DM'.

NMR spectroscopy measurements.—A Bruker PC 120 Minispec, operating at a resonance frequency of 20 MHz was used. The probehead (10 mm) was kept at 20 °C. The acquisition parameters used were: relaxation delay 2 s, 90° pulse width between 1.4 and 1.6 μs, attenuation 33. The FID signal was sampled with a Team490 acquisition card from Bakker Electronics as follows: data points: 1024; number of acquisitions: 128; time intervals: 1 and 5 μs. The signal intensities were obtained in volt/gram, but as these values will be different on different instruments, even for the same nominal signal attenuation value, the intensities are expressed in arbitrary units (a.u.).

3. Results and discussion

Comparison of literature quantitative data for starch-granule hydration is difficult since most are based on specific water properties arbitrarily settled. Thus, according to the approach used, either free and bound water, or solvent and non-solvent water is discussed.

Quantification of signals obtained from starch.—As starch is known to undergo structural modifications, notably gelatinization and retrogradation (the latter process going on over many weeks), one of the first objectives was to define a reference parameter in order to be able to compare in a quantitative manner some of the measurements taken during the intervening period. As samples were kept in sealed tubes, the only constant parameter is the total value of the signal resulting from the liquid, intermediate or amorphous, as well as solid phases. However, due to the dead-time of the coil receiver, about 10 μs, the first measurement could only be taken 11 μs after the pulse, during which time the 'solid' signal is expected to decrease by about 30–40% maximal intensity. In the case of determination of the solid/liquid ratio in fat products with the 'direct' method, the measured signal of solid phase was corrected by a factor of about 1.4 [14]. However, the value of this factor depends on various physical parameters [15] and cannot be predicted.

To determine the signal, I_{0s}, of the solid phase, a bi-Gaussian fitting [16,17], expressed by

$$I(t) = \sum_{i=1}^{2} I_i \exp\left(-\left(\frac{t}{T_{2i}}\right)^2\right) \quad \text{with } I_{0s} = \sum_{i=1}^{2} I_i, \tag{1}$$

for relaxation curves sampled every μs, from 11 μs onwards, was performed. It must be remembered that both parameters are mathematical and not physical.

Depending upon the sample, the first 15–30 measurements of the FID signal were used for the fitting of the solid phase signals (Fig. 1) in order to obtain a statistical scattering distribution of residuals ($I(t)_{calculated} - I(t)_{measured}$). A signal corresponding to the solid phase only was obtained by removing the signal due to liquid and intermediate components obtained by a multi-Gaussian fitting of the raw FID signal. It has effectively been shown [18,19], that the FID of a liquid phase obtained in an inhomogeneous magnetic field appears to be Gaussian in shape (Fig. 2).

The signal amplitude of a sample is given by the sum of populations of the components of the liquid, intermediate and solid phases; for instance with a 0.1428 g H_2O/g DM starch suspension, the following parameters have been obtained: $T_{2a} = 14.2$ μs, $I_a = 1055$ a.u., $T_{2b} = 189$ μs, $I_b = 49$ a.u., $T_{2c} = 468$ μs, $I_c = 126$ a.u., $T_{2d} = 790$ μs, $I_d = 32$ a.u. From Table 1, it can be inferred that for each of the six samples taken separately, the signal has the same value for samples in suspension, fresh gel, then retrograded gel form, with each having very different ratios of solid phase. This provides good evidence of the correctness of fit of the 'solid' signal. Moreover, these 18 values of I_0 are very closely related to the number of protons (n_H) present in the samples, as calculated from the composition and the proton density of starch (0.0617 atom gram of hydrogen, atg H/g DM) and water (0.1111 atg H/g); in addition, it should be noticed that the correlation line effectively passes through the origin with

$$I_0 = -9.16 + 16300 n_H \text{ and } r^2 = 0.9966.$$

From the slope (a.u. per atom gram of hydrogen), a theoretical signal of 1010 a.u. per gram of anhydrous starch and 1815 a.u. per gram of water was determined.
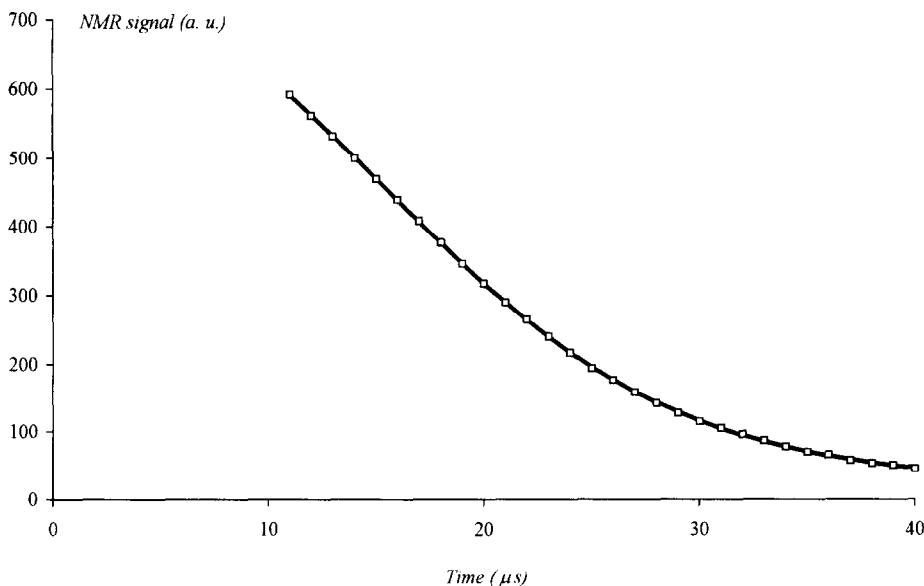

Fig. 1. Multi-Gaussian fitting of the solid phase NMR FID signal from a starch suspension at 78.2% w/w water content.
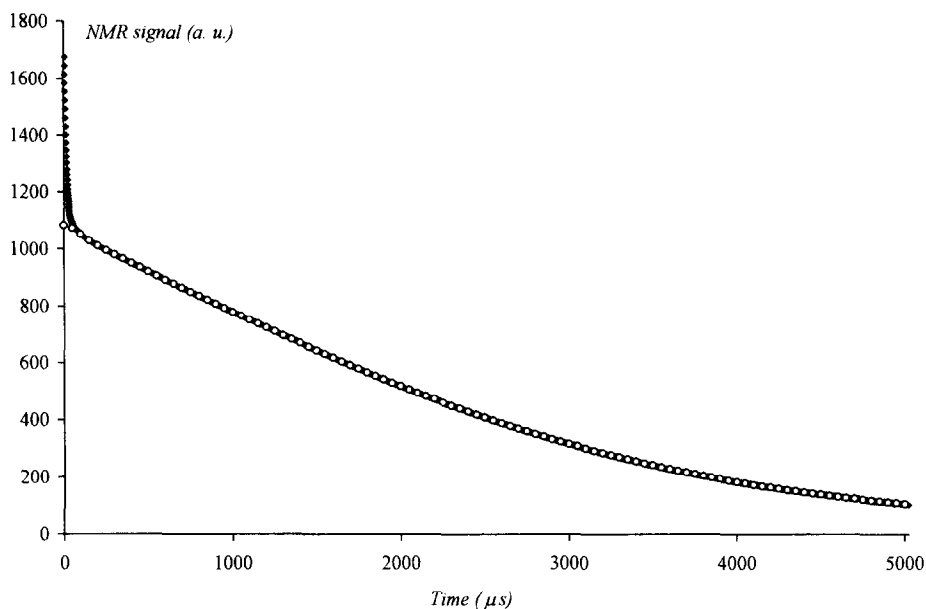
Fig. 2. Multi-Gaussian fitting of the liquid phase NMR FID signal a starch suspension at 78.2% w/w water content.

Table 1

Values obtained with a multi-Gaussian fitting of starch–water samples with various water contents and in different states (a: suspension; b: fresh gel; c: retrograded gel; S_c: solid component; I_c: intermediate component; L_c: liquid component; T_s: total signal; S/H: signal per atom gram hydrogen)

Water content (% w/w)	State	S_c (a.u./g DM)	I_c (a.u./g DM)	L_c (a.u./g DM)	T_s (a.u./g DM)	S/H [a] (a.u./atg H)
14.28	a	1055	175	32	1262	16272
	b	1050	164	27	1241	15980
	c	1054	170	25	1249	16105
29.27	a	1022	178	349	1549	16437
	b	994	220	322	1536	16312
	c	1000	200	322	1532	16259
39.93	a	799	435	514	1748	16481
	b	870	254	566	1690	15936
	c	691	524	506	1721	16235
53.68	a	781	477	749	2007	16539
	b	517	654	777	1948	16053
	c	594	602	745	1941	15990
67.64	a	901	345	946	2192	16009
	b	481	629	1082	2192	16014
	c	668	490	1032	2190	16000
81.66	a	906	360	1236	2502	16410
	b	335	677	1488	2500	16401
	c	609	505	1391	2505	16433

[a] Average = 16,215; standard deviation = 204; coefficient of variation = 1.2%.

Internal reference.—To confirm these results, it was necessary to check, using a standard, that the total signal of each sample was proportional to the amount of hydrogen atoms. As all the water present does not constitute a single phase, it was not possible to use the signal from water as a reference. In addition, it is known that the quality factor Q of the resonating circuit of the probe depends on the nature of the sample [20]. Therefore, the pulse efficiency, and the signal amplitude per atom gram of hydrogen might vary as a function of Q, making it difficult to be sure that the signal per gram of pure water could be used as an external reference for a starch–water medium. Furthermore, the signal per gram of starch was different when the sample was in a 5 mm diameter tube (1670 a.u./g) and in the 'crown' between 5 and 10 mm diameter concentric tubes (2185 a.u./g), making it impossible to use a water external reference. Thus, the use of an internal reference was preferable.

The conditions required to use the internal reference have therefore been established. According to the water content, it could be either in a state of a strong interaction with starch or partly in a strong interaction and partly in a liquid state [21]. Below 0.05 g H_2O/g DM, all water protons present had a 'solid-like' behaviour (Fig. 3). In Fig. 4, the liquid phase signal (measured at 70 μs) was plotted against the water content. A change in the slope could be seen at about 0.55 g H_2O/g DM water content. The assumption is that, under this threshold, the addition of water make some hydrogen groups or some bound water molecules more mobile. The hydrogen atoms of these entities would then result in an additional 'liquid' signal. Above 0.55 g H_2O/g DM water content, an addition of water did not modify the state of these components any more, indicating that the increase of the 'liquid' signal was only due to the added water.
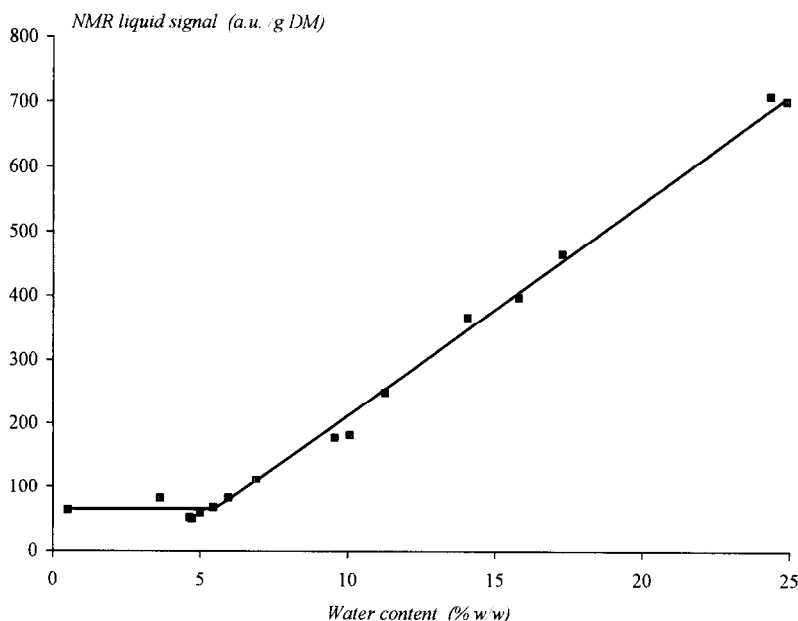

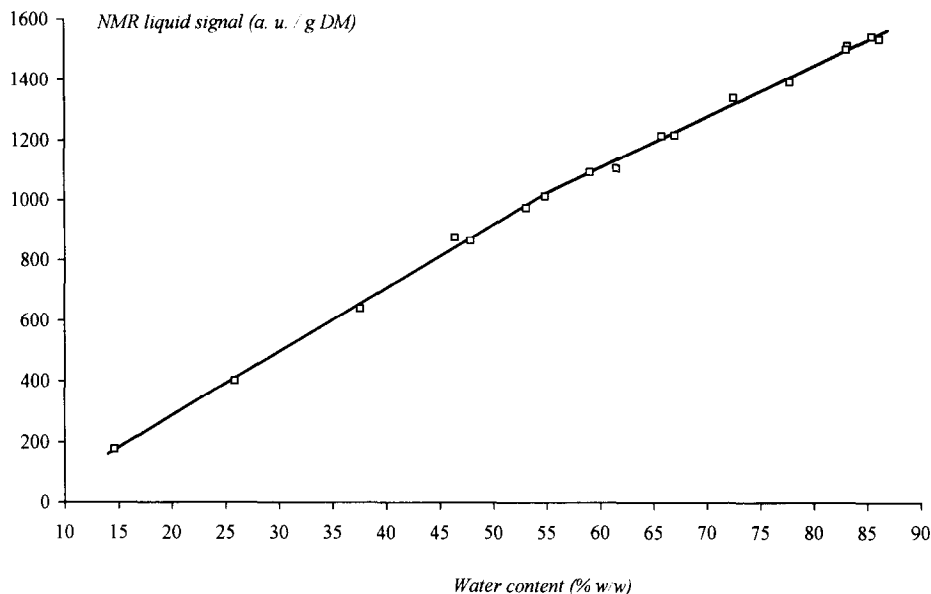Fig. 3. Evolution of the NMR spectroscopy 'liquid' signal of starch suspensions at low water content.

Fig. 4. Evolution of the NMR spectroscopy 'liquid' signal of starch suspensions according to water content.

On the other hand, when the water content increased from 0.14 to 0.55 g H_2O/g DM, the 'solid' signal value decreased from 1060 a.u. to 948 a.u. This difference can be explained by considering protons of the hydroxyl groups of starch or those of water in the monolayer in a 'solid-like' state at low water content which reaches the liquid phase at a higher water content. Besides, the 'solid' signal no longer changed above this threshold: the increase of the 'liquid' signal is then due only to the added water.

The standard addition method between 0.55 and 0.83 g H_2O/g DM thus allowed the determination of the signal for one g of water, in situ, from the slope of the straight line:

$$S_{70 \, \mu s} = f(\text{water content}),$$

where $S_{70 \, \mu s}$ is the signal sampled 70 μs after the 90° pulse, the slope = 1800 a.u., $\sigma = 44$, $r^2 = 0.963$, $n = 14$.

This value is very close to the signal obtained for a sample of distilled water, 1790 a.u. per gram, resulting in a signal of 16210 a.u. per atom gram of hydrogen, in good agreement with that obtained from the total signal of suspensions, fresh gels and retrograded gels (16300 a.u.). Thus, the accuracy and the reproducibility of the determination of the solid-phase signal was confirmed.

Solid–liquid ratio in starch–water suspensions.—The 'solid' signal of five saturated wheat starch samples (water content between 0.8 and 0.85 g H_2O/g DM) resulted in an average value of 933 a.u. ($\sigma = 9$; coefficient of deviation = 1%), while the theoretical signal of one gram of dry starch is 1010 a.u. The probability that these two values belong to the same population is not higher than 1%, indicating that the difference between these two values is highly significant. Thus, approximately 7.5% [100 × (1010

− 933)/1010] of starch gives a 'liquid' signal at saturation. This value was confirmed by a study of starch protons exchanged by deuterium in suspension in deuterium oxide, for which the presence of a 'liquid' signal is also observed at about the same proportion (7.0 and 7.3%). This 'liquid' signal probably corresponds to the end of the polysaccharide chains, which acquire mobility in suspension. It was checked that the 99.9% enriched deuterium oxide used did not result in a measurable ^{1}H NMR signal, and an evaluation of the percentage of hydrogen atoms exchanged by deuterium atoms, after each of the five cycles of exchange process (dehydration–deuterium oxide hydration) to obtain deuterated starch gave a theoretical residual amount of exchangeable OH of approximately 1%.

Quantification of the average number of hydroxyl sites exchangeable in wheat starch. —Glucosyl units of starch have a primary hydroxyl group at C-6, except when branching as in amylopectin is involved, and two secondary hydroxyl groups at C-2 and C-3. Part of these constituents are able, according to their steric environment, to exchange with the surrounding aqueous phase. Many authors have studied the reactivity of native starch granules [22–24] with various reagents: OH-2 is the most readily substituted (72–94%), then OH-3 (0–17%) and OH-6 (6–11%) [25–27]. Obviously, penetration of a reagent molecule in the starch granule is dependent upon steric hindrance. According to Biliaderis [23], acetylation of smooth pea starch takes place in the external layers of the granule whereas hydroxypropylation of corn starch is uniform throughout the granule.

The accessibility of the hydroxyl hydrogen for exchange with deuterium oxide in starch seems to be rather variable. Nara [28] reported that, among studies performed using infrared absorption spectra, some authors found that the exchangeability of hydroxyl hydrogen by deuterium approached 100%, while others found that some hydroxyl groups were not deuterated. Despite the difficulty of determining quantitatively the amount of hydroxyl hydrogen atoms exchangeable with deuterium in starch by infrared absorption spectroscopy, as discussed by Hennig [29], Nara [28] gives an accessibility factor varying between 76 and 82% for different starches, according to their botanical origins.

We propose to account for the exchangeable hydroxyl sites by comparing solids signals $(I_0 - S_{70\,\mu s})$ obtained with suspensions of anhydrous, native, and exchanged starch in water and deuterium oxide at saturation, considering the equal probability of the hydroxyl and deuterated hydroxyl exchange [30].

For a deuterium oxide starch suspension, the 'liquid' S_{liq} signal is expressed by:

$$S_{liq} = S_{fw} + S_l + S_{eOH},$$ (2)

with

S_{fw} = Signal of the free water,
S_l = Signal from 'liquid' starch,
S_{eOH} = Signal from protons of exchanged hydroxyl in 'solid' starch
and the measured 'solid' signal S_{sol} is given by the expression:

$$S_{sol} = S_s - S_{eOH}, \quad \text{with } S_s = \text{Signal from 'solid' starch.}$$ (3)

Table 2
Number of exchangeable hydroxyl sites in 'solid' starch per glucosyl unit for various water and deuterium oxide contents (S_s: solid signal; SN: signal of exchanged hydroxyls from 'solid' starch; n_e: number of exchangeable hydroxyl sites in 'solid' starch per glucosyl unit)

Water content (% w/w)	Deuterium oxide content (% w/w)	S_s (a.u./g DM)	SN (a.u./g DM)	n_e [a] (a.u./g DM)
14.28	68.16	776.7	156.0	2.22
14.28	68.84	784.2	148.5	2.09
14.28	68.25	772.6	160.1	2.29
–	87.28	732.1	200.6	2.32
14.28	82.58	762.4	170.3	2.31
14.28	84.89	744.8	187.9	2.56
–	96.62	716.7	216.0	2.48
–	68.73	735.0	197.7	2.38

[a] Average = 2.33; standard deviation = 0.14; coefficient of variation = 6%.

The number N of exchanged OH in 'solid' starch is given by:

$$N = \frac{S_{eOH}}{S_{per\,atg\,H}}, \qquad (4)$$

n_e being the number of exchangeable hydroxyl sites in the 'solid' starch, D the number of deuterons and H the number of protons coming from water. The proportion of exchanged hydroxyls is then:

$$\frac{N}{n_e} = \frac{D}{H + D + n_e} \text{ which gives } n_e = N \cdot \frac{H + D}{D - N}. \qquad (5)$$

To this value must be added the number of hydroxyl sites in 'soluble' starch, that are considered as accessible. Measurements were done on native or anhydrous starch in suspension with deuterium oxide at saturation. The results in Table 2 have been obtained by assuming that the signal per atom gram of protons is 16300 a.u. and the 'solid' signal of 1 g of starch in suspension is 933 a.u. (as determined above with five samples).

Taking into account the amount of hydroxyl protons present in the 'liquid' phase of starch (7.5% of starch), the final value is 2.55 exchanged hydroxyl groups per glucosyl residue, i.e. an average value of 85% for the 3 hydroxyl groups of the glucosyl unit. On the other hand, the total signal obtained for an exchanged starch in suspension in deuterium oxide (0.7187 g D_2O/g DM) is 734 a.u., which gives $n_e = 2.6$, i.e. 86.5%, very close to the former value. The amount of non-exchangeable hydroxyl groups would then be around 14%.

4. Conclusion

A bi-Gaussian fitting of the NMR FID signal allows an exact and repeatable quantification of the 'solid' phase signal independent of the ratio of the two phases. The standard addition method enables, within a defined range of water content, the determi-

nation of an in situ reference which is in good agreement with the result from the multi-Gaussian fitting of the FID signal and with the total number of hydrogen atoms in the samples. It is demonstrated that about 7.5% of wheat starch in suspension has a 'liquid-like' behaviour, and about 86% of the hydroxyl groups found in wheat starch are exchangeable by deuterium. Above a water content of 0.55 g H_2O/g DM, the 'solid' signal is shown to correspond only to the protons of the glucosyl units.

Acknowledgements

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