

Mobility and Distribution of Water in Cassava and Potato Starches by ^1H and ^2H NMR

PATHAMA CHATAKANONDA,[†] LEONARD C. DICKINSON,[‡] AND
 PAVINEE CHINACHOTI*[†]

Food Science Department and Department of Polymer Science and Engineering,
 University of Massachusetts, Amherst, Massachusetts 01003

Mobility and distribution of water in cassava (rainy and drought crops) and potato starches were studied by solid state and NMR relaxometry as a function of H_2O and D_2O contents ranging from 0 to 44% (dry basis). Measurements of relative mobility derived from ^2H solid state NMR were based on relative area and line shape analysis. The narrow peak (mobile component) started to show at 5% and increased with increasing D_2O content. This increase in mobile fraction was accompanied by a line narrowing. The mobile fractions of deuterons reached a >98% level above 15% D_2O , which is well below the water holding capacity of starch (~27%) and the previously assigned “glassy–rubbery transition point” (24.3%; Jouppila, K.; Roos, Y. H. The physical state of amorphous corn starch and its impact on crystallization. *Carbohydr. Polym.* **1997**, *32*, 95–104). This reconfirms the liquidlike nature of water in the so-called glassy state of starch granules. The plasticization effect of water on starch chains was observed at 14–17% for cassava and potato starches as indicated by the T_1 minimum. This, however, did not seem to relate to the difference observed in swelling among the starches studied.

KEYWORDS: Cassava starch; water mobility; NMR

INTRODUCTION

Water plays an important role in determining the functionalities of starch. Processing treatments of starch, such as heating and freezing, depend on the dynamic states of starch hydration. NMR can be used to monitor the dynamics of various water populations. Various NMR methods have been applied including solid state NMR, ^{13}C cross-polarization magic angle spinning (CP/MAS), wide-line NMR, high-resolution NMR, and cross-relaxation ^1H NMR. With these techniques, heterogeneity in the backbone and side chain mobility of starch and water can be observed. For instance, the wide-line, high-resolution NMR was used to observe rigid and mobile proton components (2).

Using ^{13}C CP/MAS NMR, a relative proportion of short-range order structures (double helices) in starches can be determined (3–5). They concluded that the proportion of double helices significantly increased with increasing water content in starch granules. It has been reported that the proportion of double helices (short-range order) was not influenced when the water was greater than 5% (6). However, crystallinity (long-range order) is found to be dependent on water content (7).

Solid state ^2H NMR is also useful in investigation of water dynamics in semisolid and solid states, such as starch granules.

The dynamics of unfreezable water was studied in waxy maize starch. This water was found to be highly mobile (liquidlike) even in solid (e.g., 9.3% D_2O) indicating that water in a glassy state can be extremely mobile (8). In potato starch, anisotropically mobile protons were observed at >35% water content (9).

In preliminary studies, functional properties of cassava starch have been investigated as a function of growth conditions (i.e., rainy or drought) and harvest times (10, 11). In this work, cassava starches from drought and rainy crops showed differences in swelling behavior and melting of amylopectin upon gelatinization in excess water. Understanding such differences is of importance to quality control in the starch industry. In this study, hydration behaviors of cassava (A type) and potato (B type) starches at low and intermediate moisture contents were studied by solid state ^2H NMR and ^1H NMR relaxometry. NMR was applied to investigate water behaviors among the starches and the relationships to their physicochemical properties.

MATERIALS AND METHODS

Sample Preparation. Cassava starch from the cultivar Kasetsart 50 (KU50) planted under the same field in May 2000 (the rainy season, “R”) and in November 2000 (the dry season, “D”) at Chachuang Soa, Thailand, was provided by Cassava and Starch Technology Research Unit, Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI). Potato starch was obtained from AVEBE (AVEBE America Inc., Princeton, NJ). Cassava and potato starch samples were dried in a vacuum oven (60 °C, 29 in Hg overnight) and equilibrated

* To whom correspondence should be addressed. Tel: 413-545-1025. Fax: 413-545-1262. E-mail: pavinee@foodsci.umass.edu.

[†] Food Science Department.

[‡] Department of Polymer Science and Engineering.

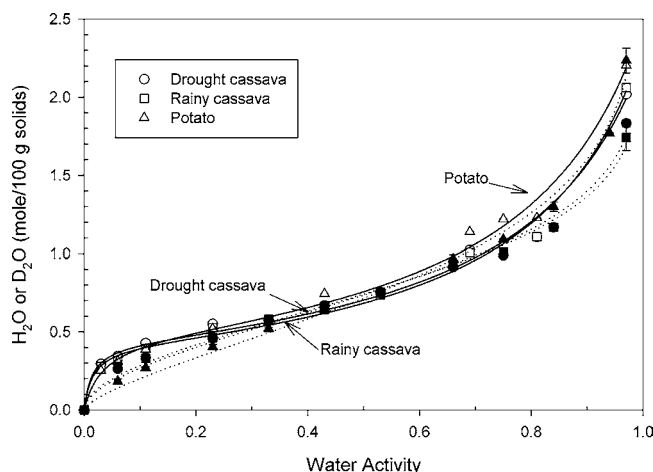


Figure 1. Moisture sorption isotherms from H₂O (closed symbols) and D₂O (open symbols) of cassava and potato starches at 25 °C. The lines represent the GAB fitted curves for H₂O (dotted lines) and D₂O (solid lines) isotherms ($R^2 = 0.99$).

by placing the samples in minidesiccators over saturated salt solutions of known water activities (ranging from 0.03 to 0.97 a_w). The saturated salt solutions were prepared with deionized distilled H₂O or 99.9% D₂O depending on the NMR experiments conducted. The samples were then incubated at 25 °C for 7–10 days to allow equilibration before the NMR analyses.

Deuterium Solid State NMR. ²H NMR determination was carried out using a DSX300 spectrometer (Bruker Instruments Inc., Billerica, MA) with a wide-line probe. A 0.2–0.3 g amount of sample was placed in a 5 mm NMR tube. The ²H NMR spectra were obtained using a (90°– t_1 –180°– t_2 acquisition) pulse sequence with a 2.0 μ s 90° pulse. The data were acquired with 150 kHz spectral width, 3 s recycle delay, and 1024 scans. Free induction decays (FIDs) obtained from a 90° pulse were analyzed using WIN NMR (Bruker Instruments Inc.) to obtain Fourier transformed spectra. Spectra with overlapping peaks were deconvoluted using Peakfit (Jandel Scientific, San Rafael, CA) to obtain the intensity (area) and line width at half-height (LW). The amount of mobile component was approximated from the relative intensity (area) of the narrow peak over the total. All samples were run in duplicate, and the experimental error was within 15%.

Proton NMR. A DPX MARAN NMR spectrometer (Resonance Instruments, Whitney, U.K.) operating at 23 MHz proton resonance frequency was used to acquire water proton transverse relaxation time. Approximately 0.5 g of each sample was placed in a 10 mm glass tube and covered with a tight cap before placing it in another 18 mm NMR tube. FIDs were obtained from a 90° pulse (4 μ s pulse width) followed by 1 μ s of dwell time and a recycle decay of 10 s. The FID envelopes were analyzed as a continuous distribution of exponentials with Resonance Instruments WINDXP software. All measurements were done in duplicate at 25 °C.

T_1 was measured by the inversion recovery (180°– τ –90°). The 90 and 180° pulse widths were 4 and 8 μ s, respectively. A recycle delay of 10 s and 10 τ values ranging from 50 μ s to 1 s was applied for each T_1 determination.

RESULTS AND DISCUSSION

Water Sorption Behavior. Starch samples were equilibrated at various water activities (a_w) over saturated salt solutions containing D₂O or H₂O. The resulting water sorption isotherms of all starch samples are shown in **Figure 1**. The sigmoidal curves were fitted according to the Guggenheim Anderson de Boer (GAB) equation (12, 13)

$$M/M_0 = (C_g K a_w) / [(1 - K a_w)(1 - K a_w + C_g K a_w)]$$

where M is the equilibrated moisture content (% dry basis) and

Table 1. Monolayer Values of H₂O and D₂O (g/100 g Solids) at 25 °C for Cassava and Potato Starches Obtained from BET and GAB Equations

starch sample	BET analysis (R^2)		GAB analysis (R^2)	
	H ₂ O	D ₂ O	H ₂ O	D ₂ O
drought cassava	7.35, 0.998	8.61, 0.999	7.93, 0.993	8.50, 0.991
rainy cassava	7.17, 0.995	8.64, 0.999	8.30, 0.995	8.17, 0.993
potato	7.34, 0.999	8.50, 0.993	7.82, 0.995	9.34, 0.991

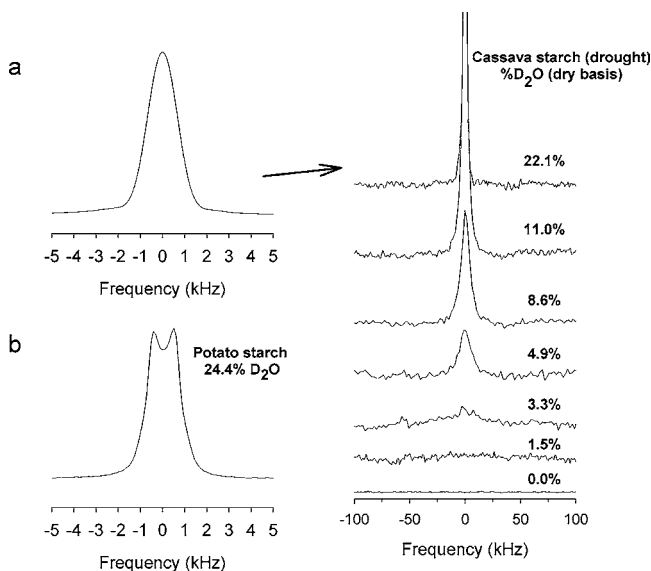


Figure 2. Narrow peaks from ²H solid state NMR spectra of (a) cassava starch (drought) and (b) potato starches hydrated with D₂O at 25 °C.

C , C_g , and K are constants. The fitting (R^2 of 0.99) gave a M_0 (“monolayer” water content) as reported in **Table 1**.

The BET (14) and GAB monolayer values were determined and are shown in **Table 1**. The GAB equation gave slightly different M_0 values from the BET equation. The BET equation focuses on surface adsorption (15), while the GAB equation takes into consideration modified properties of the sorbed water in the multilayer region (16). The BET M_0 reported in the literature for cassava starch is in the range of 5.8–6.4 g H₂O/100 g solids (17) and that for potato starch is in the range of 6.0–8.2 g H₂O/100 g solids (18). The GAB M_0 has been reported to fall in the range of 7–11% water (dry basis) for starches (19). M_0 values obtained from D₂O were slightly higher than that from H₂O isotherms (on a gram basis). The a_w values for D₂O have been shown to be slightly higher (within 5%) than those for H₂O due to the difference in their molecular weight (20).

Deuterium Solid State NMR. ²H solid state NMR spectra of drought cassava starch and potato starch containing 0–44% D₂O are shown in **Figure 2a,b**. The immobile (broad) and mobile (narrow) peaks are shown. No mobile (narrow) peak was found at low D₂O content (<5% D₂O). At $\geq 5\%$ D₂O content, the NMR spectra for all starch samples were deconvoluted into a narrow Lorentzian (more mobile) and a Gaussian (less mobile) peak. The Gaussian component was extremely broad appearing slightly above the baseline within the 144 kHz range and did not change with water content. These immobile deuterons exhibited a LW of 30–40 kHz or approximately 8–10 μ s T_2^* indicating that D₂O was present in a rigid domain. It was also possible that deuterons might have exchanged with the starch protons and deuterated starch might significantly contribute to the broad component. However, when we com-

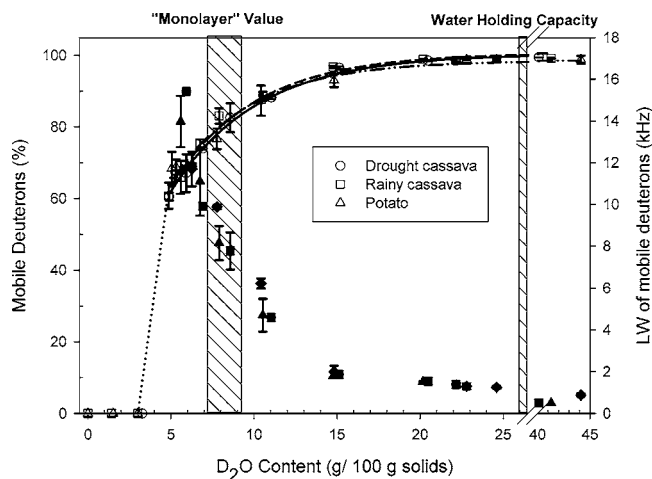


Figure 3. Fraction of mobile deuterons and corresponding line width obtained from solid state ^2H NMR experiment as a function of D_2O content.

pletely dried the sample, no Gaussian peak was found, i.e., no deuterium contribution from the starches and most signals observed were from D_2O . A similar result has been found in the case of waxy corn starch (8).

In the case of potato starch (**Figure 2b**), the narrow peak showed a Pake pattern (doublet) at above 24% D_2O . The sample above 50% D_2O showed an additional center peak possibly representing bulk water. Similar results were earlier reported by Yakubu et al. (9, 21, 22). The Pake pattern reflects an anisotropic motion of mobile water (1 kHz), whereas no Pake pattern observed in cassava starches suggests a rapid exchange among mobile populations within the NMR time frame.

No mobile deuteron was observed in the 0–4% D_2O content range (**Figure 3**). The amount of mobile deuterons increased at $\geq 5\%$ D_2O and reached a $>98\%$ level at $\sim 14\text{--}15\%$ D_2O content. At $>15\%$ D_2O content, mobile D_2O ($>90\%$) dominated the population. Assuming that the water binding capacity of starch is approximately 27% moisture, NMR results showed that the majority of the water is within starch granules.

The M_0 value from water sorption isotherms falling in the 8–9 g $\text{D}_2\text{O}/100$ g solids (**Table 1**) was higher than the 5% moisture level below which D_2O remained immobile (**Figure 3**). The first 5% of D_2O (immobile) exhibited a T_2^* of 8–10 μs . The discrepancy between NMR and water sorption isotherm monolayer values raises some concerns about the choice of methods used in determining bound water. At 8–9% D_2O , 60–80% of the deuterons were in the mobile fraction, with a T_2^* of 40 μs . The T_2^* increases by 16 times to 650 μs at 40% D_2O as the moisture content was raised to 44%.

LW decreased with increasing D_2O content as expected, from 15 kHz at 5% D_2O to 500 Hz at 44% D_2O . Line broadening and $T_2 < T_1$ at lower moisture content were results from complex phenomena, such as reduced water mobility, chemical exchange, diffusion, and chemical shift anisotropy (23, 24).

Physical State of Water and Starch Chains. At ambient temperature, it has been reported that starch was in the glassy region at $\leq 25.2\%$ water content (1). In this study, we investigated further proton T_1 and T_2 relaxations.

Starch Chain Mobility by T_1 . The ^1H NMR T_1 (spin–lattice relaxation time) of protons for cassava and potato starches was obtained as a function of water content (**Figure 4**). T_1 started at a high value ($\sim 320\text{--}350$ ms) at 0% moisture content and decreased with increasing water content to a minimum at approximately 14–17% moisture and then increased again with moisture content (**Figure 4**). Cassava starches from drought and

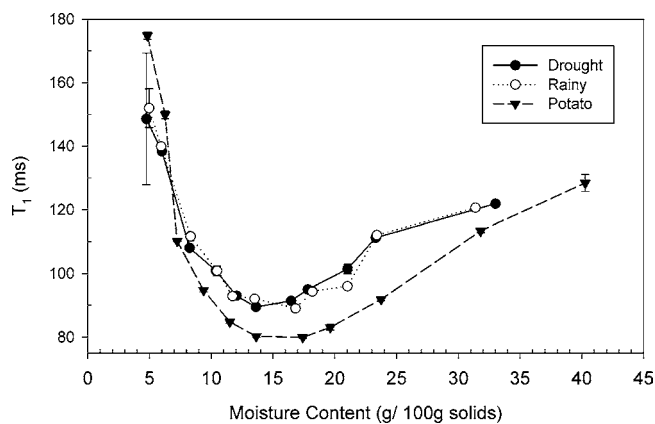


Figure 4. T_1 of protons for cassava and potato starches as a function of moisture content.

rainy seasons exhibited similar T_1 values, which were higher than T_1 for potato starch at a given moisture content. As shown in **Figure 4**, the T_1 minimum was at 14–17% water content for all samples. The T_1 minimum described an onset of molecular plasticization of starch as has been earlier reported (25, 26). For waxy corn starch, the T_1 minimum has been reported at $\sim 19.8\text{--}21.9\%$ water content (26). This onset of increase in molecular mobility moisture range was higher than M_0 from water sorption isotherms and immobile water ($\sim 5\%$ moisture content) observed by NMR.

Water Distribution by ^1H NMR (FID). Proton transverse relaxation times (T_2) for cassava and potato starches were determined by FID, and their T_2 distributions are shown in **Figure 5a,b**. Depending on moisture content, the rigid component showed T_{2S} in the range of 3–17 μs and the mobile component showed T_{2L} in the range of 110–550 μs . T_{2S} peaks remained relatively unchanged over a_w 0–0.84 but decreased in peak amplitude. T_{2L} started to appear at $\geq 5\%$ moisture (dry basis) and increased with moisture content. T_{2S} and T_{2L} as a function of moisture content are shown in **Figure 6**. Interpretation of ^1H FID data in heterogeneous system is complicated by other unknown contributions of cross-relaxation and proton exchange (27). No significant difference between cassava starch from drought and rainy seasons was observed over the range of a_w studied.

Note that no mobile component (T_{2L}) was observed at a moisture content below 5%, and all protons appeared solidlike with $T_2 \sim 10$ μs (**Figure 6**). There was some similarity in this trend as compared with the ^2H NMR data reported in **Figure 3** suggesting that T_{2L} might be more dominated by water protons. On the other hand, the T_{2S} fraction contributed by rigid protons was more dominated by the rigid starch chains. Its decrease in peak amplitude might have been at least in part affected by the plasticization of the starch chains. This seemed to occur gradually over a wide range of moisture content as observed in T_1 , i.e., all T_1 curves in **Figure 4** show a broad T_1 minimum range over moisture ranging from 10 to 20%.

This work shows that ^1H NMR can detect molecular plasticization. It is also evident that the molecular motions of starch and water are decoupled. At a moisture content below the onset of mobilization (i.e., 14–17% moisture where T_1 minimum was observed, **Figure 4**), the water mobility could be very high (mobile fraction with T_2^* ranging from 40 to 180 μs over 8–15% moisture).

With respect to the monolayer value (M_0), both ^1H and ^2H NMR experiments strongly showed that water in a 0–5% moisture content range was immobile (well below M_0 obtained

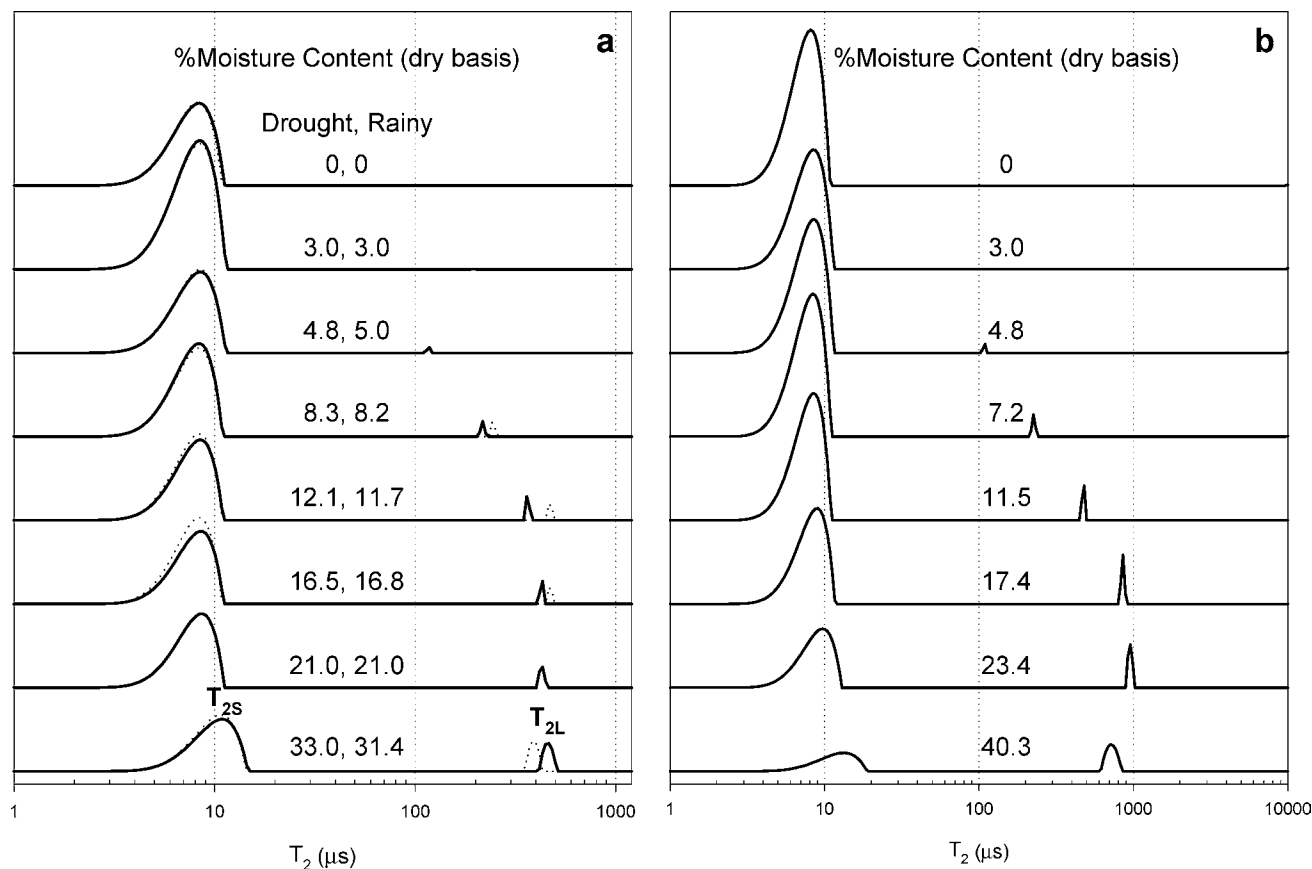


Figure 5. T_2 distribution of protons from FID experiment for (a) cassava starch from drought (dotted lines) and rainy (solid lines) seasons and (b) potato starch.

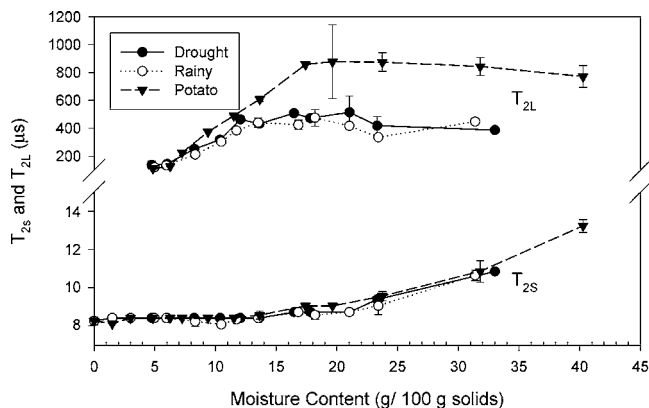


Figure 6. Transverse relaxation times (T_2) from FID for less mobile (T_{2S}) and more mobile (T_{2L}) components of cassava and potato starches as a function of moisture content.

from isotherm calculation). This immobile water may be associated with hydration of rigid starch domains and/or starch crystalline regions. This raises a question about whether this water population is in anyway related to water of crystallinity. The answer is no for the following reasons.

The water in the crystalline region has been suggested to make up 4–7% and 25–27% of the weight of A and B polymorphs, respectively (28, 29), while the rest of the water distributes in the noncrystalline (amorphous) regions of the granules. Assuming 38 and 28% crystallinity for cassava and potato starches (30), water in crystalline structures in cassava and potato starches can be estimated to be 1.5–2.7 and 7.0–7.6% of dry weight of starch granules, respectively. However, both solid state ^2H and ^1H FID experiments confirmed that water in all starches studied here was immobile at $\leq 5\%$ moisture (dry basis) and this is not

related to the levels of water of crystallinity in these starches. The amount of immobile water determined by NMR fell above the amount of structural water required for A type starch but below that for B type starch.

It has been earlier suggested that the mobility of “structural” water in A and B polymorphs is different (22). The A type crystal structure consists of a “tightly bound” component, whereas the B polymorph also contains anisotropic “weakly bound” water in addition to the tightly bound water. This weakly bound water in starch is probably located in channels within the crystallites and is more mobile than tightly bound water (31). This might explain the longer T_{2L} of potato starch, which contains a more open structure of double helices in the B polymorph, as compared to the A type polymorph in cassava starch.

It remains unclear why both A and B type starches studied here showed the same amount of immobile water. There are some possible explanations. One is that immobile water is associated with some locations or sites in starch that do not allow the water to exchange rapidly with other water populations. As water was removed from starch below 5% moisture content, starch crystals increasingly lost its crystallinity and eventually completely destroyed at 0% moisture. In such a case, water could be tightly bound to $-\text{OH}$ groups of starch and become immobilized in the rigid (and amorphous) starch domains.

Another explanation is that the data obtained might have been manifested by a strong quadrupolar interaction (in ^2H NMR) and dipole–dipole interaction (in ^1H NMR) leading to an extreme line broadening and loss of the most rigid signal. However, in the case of ^2H NMR, the experiment was repeated with dead time reduced from 10 to 5 μs and a slightly more

rigid signal (by 10%) was observed. This indicated the undetectable fraction of some immobile signal, which is considered to be very rigid ($T_2^* < 10 \mu\text{s}$) and weak.

In summary, ^2H solid state NMR spectra and T_2 distribution of protons in cassava (from drought and rainy crops) and potato starches showed undetectable signals at $< 5\%$ moisture, which was not corresponding to the level of crystalline water nor monolayer values from water sorption isotherms. The immobile water was in a rigid domain. The plasticization effect of water on starch chains was observed at 14–17% moisture (dry basis) for cassava and potato starches as indicated by the T_1 minimum. Water mobility was high ($T_2^* > 40 \mu\text{s}$) at $\geq 5\%$ water (dry basis) where starch and water mobility were decoupled. No effect of drought and rainy seasons on hydration properties of cassava starches was seen within the range of moisture content studied. The Pake pattern at $\geq 24\%$ moisture (dry basis) in potato starch occurred as moisture increased close to water holding capacity (water saturation) of starch granules, suggesting a beginning of spatial separation between water inside and outside of starch granules.

ACKNOWLEDGMENT

Access to the NMR facility (Department of Polymer Science and Engineering, University of Massachusetts) and technical support for NMR analysis are appreciated. We also thank Cassava and Starch Technology Research Unit, Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), for providing cassava starch samples.

LITERATURE CITED

- Jouppila, K.; Roos, Y. H. The physical state of amorphous corn starch and its impact on crystallization. *Carbohydr. Polym.* **1997**, *32*, 95–104.
- Wu, J. Y.; Bryant, R. G.; Eads, T. M. Detection of solidlike components in starch using cross-relaxation and Fourier transform wide-line ^1H NMR methods. *J. Agric. Food Chem.* **1992**, *40*, 449–455.
- Gidley, M. J.; Bociek, S. M. Molecular organization in starches: A ^{13}C CP/MAS NMR study. *J. Am. Chem. Soc.* **1985**, *107*, 7040–7044.
- Cooke, D.; Gidley, M. J. Loss of crystalline and molecular order during Starch gelatinization: Origin of the enthalpic transition. *Carbohydr. Res.* **1992**, *227*, 103–112.
- Morgan, K. R.; Furneaux, R. H.; Larson, N. G. Solid-state NMR studies on the structure of starch granules. *Carbohydr. Res.* **1995**, *276*, 387–399.
- Bogacheva, T. Y.; Wang, Y. L.; Hedley, C. L. The effect of water content on the ordered/disordered structures in starches. *Biopolymers* **2001**, *58*, 247–259.
- Buleon, A.; Bizot, H.; Delage, M. M.; Pontoire, B. Comparison of X-ray diffraction patterns and sorption properties of the hydrolyzed starches of potato, wrinkled and smooth pea, broad bean and wheat. *Carbohydr. Polym.* **1987**, *7*, 461–482.
- Li, S.; Dickson, L. C.; Chinachoti, P. Mobility of “unfreezable” and “freezable” water in waxy corn starch by ^2H and ^1H NMR. *J. Agric. Food Chem.* **1998**, *46*, 62–71.
- Yakubu, P. I.; Baianu, I. C.; Orr, P. H. Unique hydration behavior of potato starch as determined by deuterium nuclear magnetic resonance. *Carbohydr. Polym.* **1990**, *6*, 289–306.
- Santisopasri, V.; Kurotjanawong, K.; Chotineeranat, S.; Piya-chomkwan, K.; Sriroth, K.; Oates, C. G. Impact of water stress on yield and quality of cassava starch. *Ind. Crops Prod.* **2000**, *13*, 115–129.
- Sriroth, K.; Santisopasri, V.; Petchalanuwat, C.; Kurotjanawong, K.; Piya-chomkwan, K.; Oates, C. G. Cassava starch granule structure-function properties: Influence of time and conditions at harvest on four cultivars of cassava starch. *Carbohydr. Polym.* **1999**, *38*, 161–170.
- Van den Berg, C. Development of B. E. T.-like models for sorption of water on foods, theory and relevance. In *Properties of Water in Foods*; Simatos, D., Multon, J. L. Eds.; Martinus Nijhoff Publishers: Dordrecht, The Netherlands, 1985; pp 119–131.
- Wolf, W.; Spiess, W. E. L.; Jung, G. Standardization of isotherm measurements. In *Properties of Water in Foods*; Simatos, D., Multon, J. L., Eds.; Martinus Nijhoff Publishers: Dordrecht, The Netherlands, 1985; pp 661–679.
- Brunauer, S.; Emmett, P. H.; Teller, E. Adsorption of gases in multimolecular layers. *J. Am. Chem. Soc.* **1938**, *60*, 309–319.
- Bell, L. N.; Labuza, T. P. In *Moisture Sorption: Practical Aspects of Isotherm Measurement and Use*, 2nd ed.; American Association of Cereal Chemists, Inc.: St. Paul, MN, 2000.
- Kapsalis, J. Influences of hysteresis and temperature on moisture sorption isotherms. In *Water Activity: Theory and Application to Food*; Rockland, L. B., Beuchet, L. K., Eds.; Marcel Dekker: New York, 1987.
- Sanni, L. O.; Atere, C.; Ayoade, K. Moisture sorption isotherms of fufu and tapioca at different temperatures. *J. Food Eng.* **1997**, *34*, 203–212.
- Chilton, W. G.; Collison, R. Hydration and gelation of modified potato starches. *J. Food Technol.* **1974**, *9*, 87.
- Iglesias, H. A.; Chirife, J. *Handbook of Food Isotherms*; Academic Press: New York, 1982.
- Kou, Y.; Schmidt, S. J. Vapor pressure and water activity measurements of saturated salt solutions made with D_2O at 20 °C. *Food Chem.* **1999**, *66*, 253–255.
- Yakubu, P. I.; Baianu, I. C.; Orr, P. H. Deuterium nuclear magnetic resonance studies of potato starch hydration. In *Water Relationships in Foods*; Slade, H., Levine, L., Eds.; Plenum Press: New York, 1991; pp 585–579.
- Yakubu, P. I.; Ozu, E. M.; Baianu, I. C.; Orr, P. H. Hydration of potato starch in aqueous suspension determined from nuclear magnetic resonance. *J. Agric. Food Chem.* **1993**, *41*, 162–167.
- Canet, D.; Robert, J. B. Time scales in NMR: Relaxation phenomena in relation with molecular reorientation. In *Dynamics of Solutions and Fluid Mixtures by NMR*; Delpuech, J. J., Ed.; J. Wiley and Sons: Chichester, 1995; pp 19–72.
- Delpuech, J. J. Time scale in NMR: Nuclear site exchange and dynamic NMR. In *Dynamics of Solutions and Fluid Mixtures by NMR*; Delpuech, J. J., Ed.; Wiley & Sons: Chichester, 1995; pp 73–172.
- Tanner, S. F.; Hills, B. P.; Parker, R. Interactions of sorbed water with starch studied using proton nuclear magnetic resonance spectroscopy. *J. Chem. Soc., Faraday Trans.* **1991**, *87*, 2613–2621.
- Kou, Y.; Dickinson, L. C.; Chinachoti, P. Mobility characterization of waxy corn starch using wide-line ^1H Nuclear Magnetic Resonance. *J. Agric. Food Chem.* **2000**, *48*, 5489–5495.
- Schmidt, S. J.; Lai, H. Use of NMR and MRI to study water relations in foods. In *Water Relationships in Foods*; Levine, H., Slade, L., Eds.; Plenum Press: New York, 1991; pp 405–452.
- Imberty, A.; Perez, S. A revisit to the three-dimensional structure of B-type starch. *Biopolymers* **1988**, *27*, 1205–1221.
- Imberty, A.; Chanzy, H.; Perez, S. The double-helical nature of the crystalline part of A-starch. *J. Mol. Biol.* **1988**, *201*, 365–378.
- Zobel, H. F. Molecules to granules: A comprehensive starch review. *Starch* **1988**, *40*, 44–50.
- Tang, H. R.; Brun, A.; Hills, B. P. A proton NMR relaxation study of the gelatinization and acid hydrolysis of native potato starch. *Carbohydr. Polym.* **2001**, *46*, 7–18.

Received for review February 13, 2003. Revised manuscript received September 1, 2003. Accepted September 18, 2003. This work was supported by the Royal Thai Government and the Reverse Brain Drain Project, National Science and Technology Development Agency (NST-DA), Ministry of Science, Technology and Environment, Thailand.