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The influence of time and conditions of harvest on the functional behaviour of cassava starch—a proton NMR relaxation study

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Abstract

The extent of irrigation of crops in the field not only affects crop yield but also the functionality of the harvested product. This irrigation effect severely affects the processing response of starch harvested from Cassava and leads to industrial quality control problems. In this paper we show how the NMR transverse proton relaxation spectrum is a sensitive probe of the effect of irrigation on cassava starch functionality. The results suggest that increased irrigation results in a looser packing of the amylose and amylopectin chains in the cassava starch granule that facilitates their plasticization and gelatinization.

Keywords: Cassava starch; Irrigation; NMR transverse proton relaxation spectrum

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1. Introduction

Cassava starch is used extensively in industry because of its unique thickening properties, its high purity, low cost and its ability to form clear viscous pastes. Unfortunately, starch functionality displays unpredictable variation, depending on the environmental conditions at the time of harvest (Asaoka, Blanshard, & Richard, 1991, 1992; Defloor, Dehing, & Delcour, 1998a; Defloor, Swennen, Bokanga, & Delcour, 1998b; Moorthy & Ramanujam, 1986; Sriroth, Santisopasri, Kurotjanawong, Piyachomkwan, & Oates, 1998a; Sriroth et al., 1998b). This variability can cause difficulties in optimizing the commercial viability of cassava starch, especially in countries like Thailand, where more than a million hectares are planted annually for the cassava industry.

In a recent study Sriroth et al. (1999) showed that the amount of rain during crop growth affects the starch pasting temperature, which increases during the dry season and decreases during the wet season. The age of the crop also affects starch quality. The cassava roots are actually ripe after 12 months, but they are often left unharvested until 14–16 months. This delay results in an increase in the starch content and a decrease in water content, but this advantage is offset to some extent by the increased fiber content, which makes the starch more difficult to extract. The starch granules from older cassava roots are also characterized by a decreased amylose content and an altered granule size distribution, gradually changing from a normal to bimodal distribution with increased harvest time. The environmental conditions also alter the response of the granules to water uptake (swelling power) and subsequent thermal gelatinization though why this is the case is unknown.

Clearly, an understanding of the effect of the field environment on cassava starch quality requires a deeper understanding of the effect of irrigation and harvesting time on the amount and distribution of water within the granules and how this influences starch biosynthesis and hence granule microstructure and the processing response. These changes suggest the use of NMR proton relaxation methods as we have already shown that these are sensitive to the (changing) microscopic distribution of

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water within the granules of potato, corn and pea starch (Hills, Godward, Manning, Biechlin, & Wright, 1998; Tang, Godward, & Hills, 2000; Tang, Brun, & Hills, 2001). In this paper we therefore use NMR relaxometry to compare the response of four samples of cassava starch to thermal gelatinization and acid hydrolysis (Lintnerization). The four samples differ in their harvest time (6 and 12 months) and in whether they have been harvested in the rainy or dry seasons.

2. Experimental

2.1. Sample preparation

Cassava plants of the cultivar Kasetsart 50 (KU 50) were planted under identical field conditions in June 1996 (the rainy season, denoted 'R') and in October 1996 (the dry season, denoted 'D') at Nakorn Ratchasrima, Thailand. Cassava roots were manually harvested after 6 and 12 months and the starch extracted within 12 h of harvesting by water extraction. The four samples were therefore denoted R6, R12, D6 and D12.

2.2. Heating stage microscopy

The thermal response (swelling and gelatinization) were directly observed using an optical microscope fitted with a 40 × lens and a heating stage (Interface Techniques Company, Cambridge, MA). A 0.5% w/w starch suspension in water under a glass cover slip was heated from 35 to 75 °C at a heating rate of 10 °C/min. Images were analysed with Imagepro Plus 3.0. The temperature dependence of the mean granule size is plotted in Fig. 1, where the mean size is defined in Image-Pro plus as the average diameter of lines drawn at 2 degree orientation increments through the object centroid.

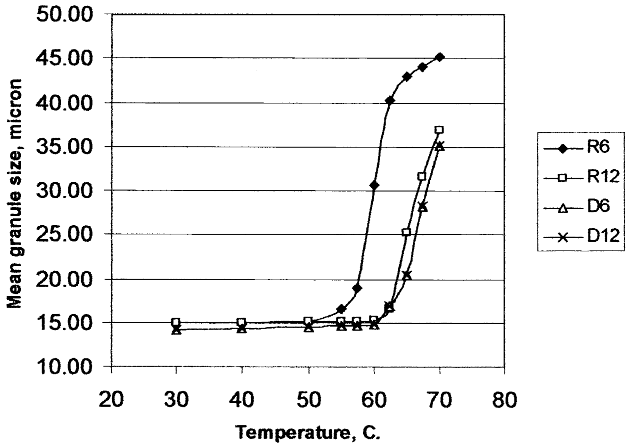

Fig. 1. Temperature dependence of the mean granule size derived from optical micrographs.

2.3. NMR characterization

The response of the starch granules to thermal gelatinization, freeze-thawing and acid hydrolysis (Lintnerization) were monitored using NMR proton relaxometry. Samples were prepared as the water-saturated packed bed of the native granules where excess water over the packed bed was removed by pipetting before NMR acquisition. In some samples the changing dynamics of the amylopectin and amylose chains was examined by removing the NMR proton signal of the water. This was done by repeated (three times) suspending the native granules in D_2O, centrifuging and removing the excess D_2O before finally preparing the packed bed in neat D_2O.

NMR gelatinization experiments were performed by gradually heating the water-saturated beds from 296 to 363 K in the NMR probe. Freezing experiments were performed by rapidly freezing the water-saturated packed beds in liquid nitrogen and then gradually raising the temperature in the NMR probe. The effects of acid hydrolysis were examined using the standard Lintnerization procedure, viz. exposure to 2.2 N HCl at 35 °C for specified times up to 32 days. After treatment the granules were washed several times in water until neutral pH was reached. The samples were then once more examined as the water-saturated packed bed.

All NMR measurements were made on a Bruker MSL100 spectrometer working at a spectrometer frequency of 100.13 MHz. 90° pulse durations were set to 2 μs with an FID (Free Induction Decay) of 4 μs dwell time. Transverse relaxation times were measured with the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence with full phase cycling and a fixed 90–180° pulse spacing of 150 μs. A recycle delay of 10–15 s was used to avoid saturation and 100 acquisitions were accumulated. The FID and CPMG echo decay envelopes were analysed as a continuous distribution of exponentials with Resonance Instruments WINDXP software (Resonance Instruments Ltd., Oxfordshire, UK). To avoid over processing data, the regularization parameter in this inversion was determined from the baseline noise. Temperature was thermostated in the usual way either by passage of air through the probe or by the evaporation of liquid nitrogen.

For ease of comparison the FID and CPMG relaxation time distributions were plotted so that the height of the largest peak was set to unity.

3. Results and discussion

3.1. Non-NMR characteristics

The main results from the non-NMR methods are summarized in Table 1. The first row in Table 1 shows that the integrated rainfall for the four cultivars during their growing period is in the expected order for rainy and dry

Table 1
Hydration properties of the four cassava starch samples

	D6	D12	R6	R12
Total rain (mm)[a]	158	895	970	1270
Average granule size (μ)	14.2	14.2	15.0	15.0
Amylose content (%)[b]	22.3 ± 0.05	17.6 ± 0.6	19.1 ± 0.9	20.7 ± 0.4
Amylose chain length (DP*n*)	3438 ± 61	4417 ± 77	2985 ± 228	2055 ± 13
Peak relative viscosity[b]	369 ± 5	381 ± 13	443 ± 14	402 ± 18
Onset gelatinization temperature (°C)[b]	65.3 ± 1.0	67.0 ± 0.9	63.4 ± 1.0	64.4 ± 1.5
Peak gelatinization temperature (°C)[b]	72.7 ± 0.9	71.9 ± 0.5	67.6 ± 0.6	68.2 ± 0.8

(±) values are the mean of three determinations.

[a] Data obtained from Sriroth et al. (1998a).

[b] Data obtained from Sriroth et al. (1999).

seasons. It is also clear from Table 1 that the different field conditions give rise to significantly different starch granule characteristics. In particular, the two rainy season samples have larger average granule sizes, lower gelatinization temperatures and higher peak paste viscosities than the drought season samples. Fig. 1 shows the results of the optical heating stage experiment. The two rainy season samples clearly have lower gelation onset temperatures than the two dry samples with R6 having the lowest onset temperature. There is a significant difference in the amylose content between the samples at a given harvest time. Amylose content was higher at 6 month than 12 month harvest time in the drought crops, but the same in the rainy crops. The mean amylose chain length (DP) in the rainy season samples is significantly shorter than for the dry season samples. In particular, the R12 mean chain length is less than half that of the D12 sample.

3.2. The effect of gelatinization on water distribution and starch chain mobility

Fig. 2 shows the distribution of proton transverse relaxation times (the 'relaxation spectrum') derived from the FID (Free Induction Decay) for a packed bed of the R6 granules. In this sample the water has been replaced by D_2O so that the observed relaxation spectrum pertains to the mobility of the non-exchanging starch CH chains in the granule. The assignment of the relaxation peaks in Fig. 2 has been discussed in an earlier paper on potato starch (Tang et al., 2000). The peak at ca. 10 μs arises from rigid amylopectin in the semi-crystalline lamellae while the longer relaxation time peak at ca. 1 ms has been assigned to a more mobile fraction of amylopectin in the amorphous regions of the granule. The same peaks have been observed in waxy maize starch, which lacks amylose, so both peaks must arise from the amylopectin rather than the amylose component. As the temperature is increased the area of the relaxation peak at ca. 10 μs decreases relative to the more mobile fraction at 1 μs and this reflects a plasticizing transition in the amylopectin which is more clearly seen in Fig. 3. This transition has already been reported for potato starch (Tang et al., 2000). What has not been reported previously is the effect of differing environmental conditions on this plasticizing transition. In particular, Fig. 3 shows that harvesting in the dry season (samples D6 and D12) shifts the transition to significantly higher temperatures, suggesting that the lower environmental water availability results in a more rigid packing of the amylopectin chains in the semi-crystalline lamellae inside the granules.

Fig. 4 shows representative relaxation spectra for the D6 cassava starch sample in D_2O measured on a longer timescale with the CPMG sequence as the sample is gelatinized. The shortest relaxation time peak at about 1 ms has been previously assigned to amylopectin in the amorphous regions of the granule and is the same as the peak near 800 μs in Fig. 2. The slight shift to 1 ms in Fig. 4 compared to the FID data in Fig. 2 is expected because the enhanced relaxation effects of diffusion through field inhomogeneities are removed by the CPMG sequence. The middle peak at about 20 ms in Fig. 4 has been assigned to mobile amylopectin, most probably in the amorphous

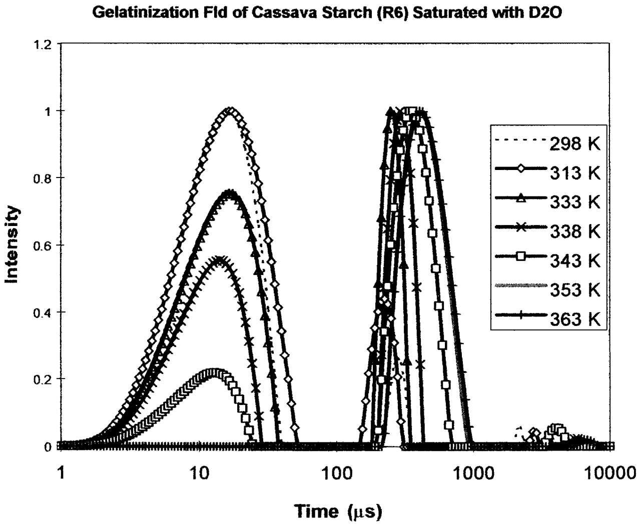

Fig. 2. The relaxation spectrum of cassava starch (R6) in D_2O measured from the FID at the indicated temperatures. The peaks have been previously assigned to a more rigid component of amylopectin in the semi-crystalline lamellae (left peak) and to a more mobile amylopectin peak, possibly in the amorphous regions of the granule (right).

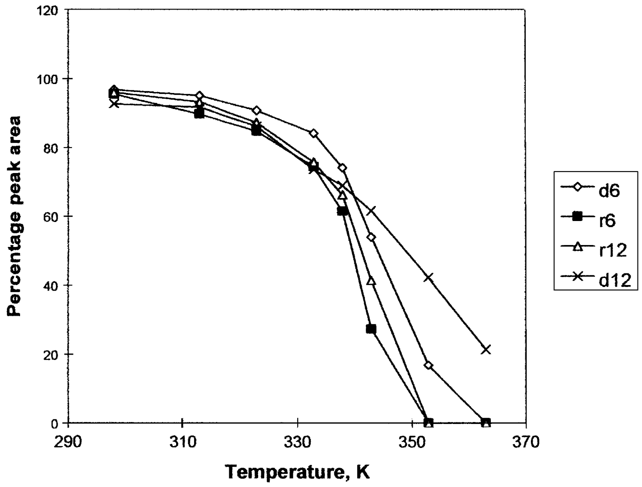

Fig. 3. A comparison of the temperature dependence of the relative peak area of the semi-crystalline amylopectin peak at ca. 10 μs in Fig. 1.

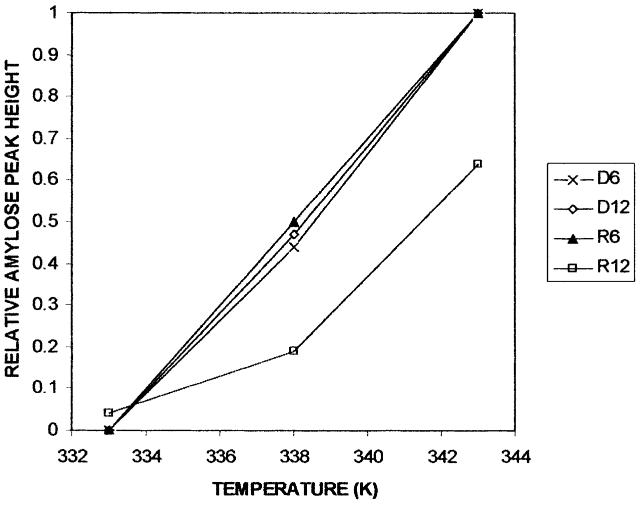

Fig. 5. The temperature dependence of the amylose peak height in the CPMG relaxation spectrum of the indicated starches in D_2O.

growth rings, while the longest relaxation time peak at ca. 80 ms arises from amylose, since it is not seen in waxymaize starch which lacks amylose (Tang et al., 2000, 2001). Fig. 4 shows that increasing temperature decreases the relative amount of amylopectin in the more rigid 1 ms component while increasing that in the more mobile amorphous regions. At the same time the relative amount of mobile amylose increases. These changes are therefore sensitive indicators of the increasing mobility of the starch chains as they experience plasticization and gelatinization. Fig. 5 plots the increase in amylose peak height as the four cassava starch samples are gelatinized. It is obvious that R12 is significantly different from the other three samples, including the R6 sample; and this difference is also reflected in the corresponding plot of the relative height of the amylopectin peak at 1 ms in Fig. 6. The shorter mean amylose chain length in the R12 sample (Table 1) is the most likely cause of this exceptional behaviour. It appears that the shorter chain length destabilizes the amylopectin causing it to be more easily plasticized and gelatinized. If so, the very rigid amylopectin corresponding to the 10 μs peak in the FID relaxation spectrum (Fig. 2) is contributing to an increase in the more mobile amylopectin peak at 1 ms in the CPMG spectrum in Fig. 4, and this has the effect of raising the peak height at 1 ms in Fig. 6 and decreasing the relative heights of the amylose peak in Fig. 5.

A direct comparison of the D_2O-exchanged proton relaxation spectra for the driest (D6) and wettest (R12) samples at 333 K, just below the gelatinization temperatures, reveals more subtle differences in the amylopectin and amylose chain dynamics. For example, Fig. 7 shows that the D6 sample not only has a larger fraction of more rigid starch protons but also an additional ‘rigid’ amylopectin fraction having a relaxation time of ca. 100 μs not seen in the R12 (or R6) samples. The relaxation spectrum on the longer

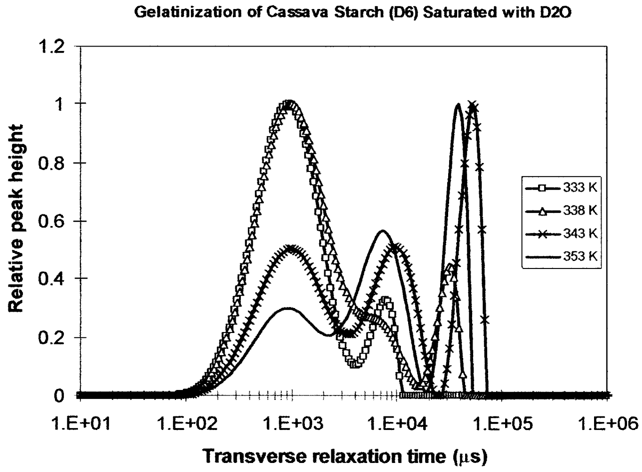

Fig. 4. Representative transverse relaxation time spectra for cassava starch (D6) in D_2O measured with the CPMG sequence at the indicated temperatures. The left peak corresponds to that on the right in Fig. 2. The middle peak to amylopectin in the amorphous growth rings and the right peak to amylose.

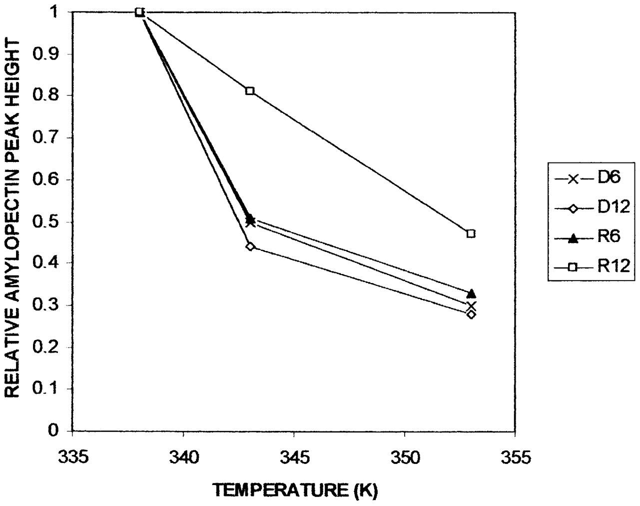

Fig. 6. The relative peak height of the amylopectin peak at ca. 1 ms in the CPMG relaxation spectrum of the indicated starches in D_2O.

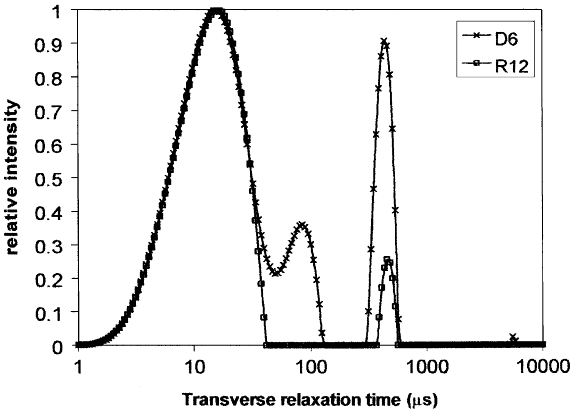

Fig. 7. Comparison of the relaxation spectra corresponding to the amylopectin chain mobility at 333 K determined from the FID of the pack granule beds in D_2O.

CPMG timescale (Fig. 8) further reveals that the R12 sample has an additional, highly mobile starch fraction with a relaxation time of ca. 100 ms, most probably arising from a mobile amylose component not seen in the D6 sample. The main amylose peak at ca. 10 ms is also significantly more mobile than that in the D6 sample, as shown by its longer transverse relaxation time. These data provide direct confirmation that the starch chains in the more irrigated R12 sample are significantly more mobile at 333 K than those in the D6 sample.

Fig. 9 shows representative CPMG relaxation spectra for the water (H_2O)-saturated packed granule bed of the D12 cassava starch. The peaks have been assigned in earlier papers and correspond to water inside the granules (the shortest relaxation time peak at ca. 3 ms) and water outside the granules (the main middle peak at ca. 20 ms). The longest relaxation time peak at ca. 100 ms only appears after partial gelatinization and presumably arises from amylose gel in certain parts of the sample. There is not sufficient water in the packed bed to permit complete, homogeneous, gelatinization, so the outside regions of the sample experience gelatinization first and this creates a heterogeneous distribution of gel and non-gelled regions. For this reason the temperature was not extended above 380 K. Even so, there are a number of clear trends in the data. Fig. 10 plots the relative peak height corresponding to water inside the granules and shows that, unlike the other samples, the D6 granules show greater swelling in the early stages of heating. This may well reflect the more difficult gelatinization (Fig. 1) that necessitates larger amounts of plasticizing water. D6 is also the last sample to gelatinize (Fig. 11) as seen in plots of the peak height of the amylose gel component.

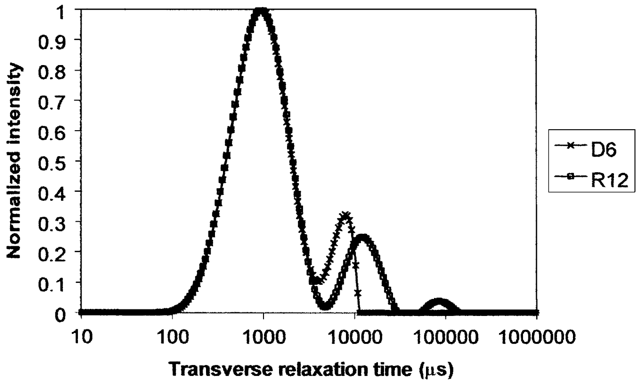

Fig. 8. Comparison of the longer timescale relaxation spectra corresponding to the more mobile at 333 K determined from the proton CPMG of the packed granule beds in D_2O.

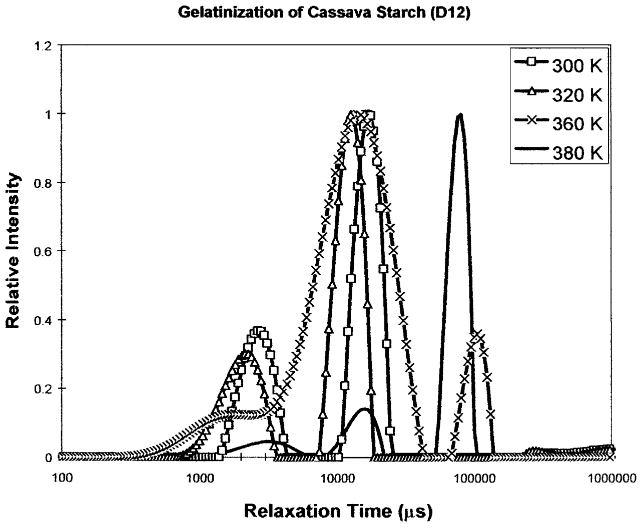

Fig. 9. The relaxation spectrum for a saturated bed of cassava starch (D12) in H_2O measured with the CPMG sequence. The three peaks from left to right have been assigned to water inside the granule (left); water outside the granule (middle) and amylose gel (right), respectively.

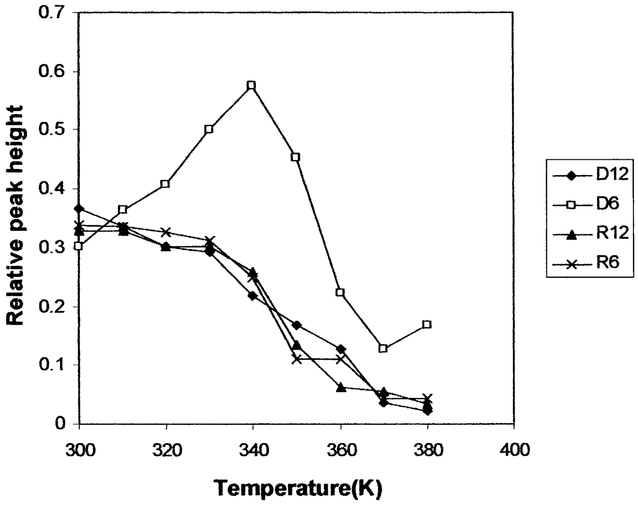

Fig. 10. The temperature dependence of the relative peak height of the ca. 3 ms CPMG peak in Fig. 9 and corresponding to water inside the granules.

3.3. NMR studies of the freeze-thawing behaviour

Fig. 12 shows the proton relaxation spectrum for the water-saturated packed beds frozen at 250 K as measured by the CPMG sequence. The shortest relaxation time peak

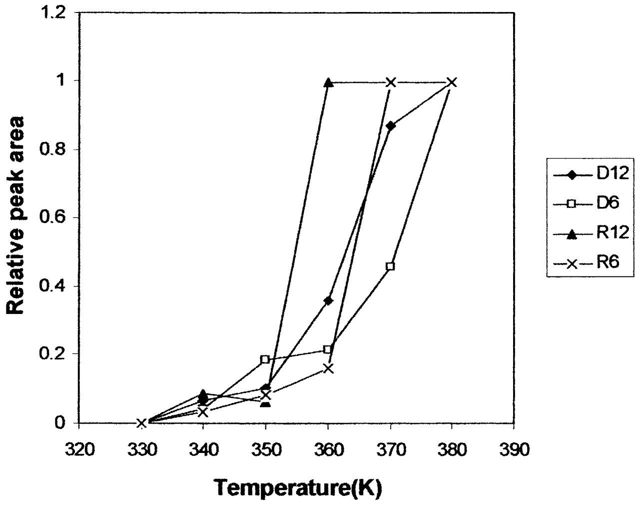

Fig. 11. The temperature dependence of the relative peak height of the ca. 100 ms CPMG peak in Fig. 9 assigned to amylose gel outside the granules.

corresponds to the more mobile starch protons while the longer peak at ca. 3 ms corresponds to non-freezing water. This assignment is based on the observation that it shifts to shorter relaxation times and decreases in area as the temperature is lowered, eventually vanishing at temperatures below 230 K. The D12 sample is an exception in Fig. 12 in that it has a much smaller contribution from the more mobile starch fraction compared to the other starches and this is consistent with the earlier observation that the D12 sample appeared to consist of more rigidly packed amylopectin.

It is interesting to note that cooling the water-saturated samples to just above freezing (277 K) results in the splitting of the peak at 3 ms (corresponding to water inside the granules) into two in the R12 sample but not in the others (see Fig. 13). A similar splitting had been observed previously for large potato granules where the two peaks resulted from slow exchange of water between the amorphous growth rings and the semi-crystalline lamellae. This is consistent with the larger average size of the R12 granules (Table 1) with correspondingly larger amorphous growth rings.

3.4. NMR studies of the effect of acid hydrolysis (Lintnerization)

Fig. 14 shows the water proton relaxation time spectrum measured by the CPMG sequence for the starches after they have been subjected to 8 days of acid hydrolysis (Lintnerization), washed and examined as a water-saturated packed bed at 298 K. It is especially noteworthy that the two rainy season starches differ qualitatively in their behaviour to the two dry season starches. The rainy season starches exhibit two well-resolved peaks and the longest relaxation time peak, corresponding to water outside the granules, is significantly longer than that of the dry season samples, especially for R12 and suggests that the rainy season starches are more susceptible to acid hydrolysis than the more compact dry season granules.

3.5. The gelatinization of the partially acid hydrolysed starches

Fig. 15 compares the gelatinization behaviour of the four starches after they have been exposed to the Lintnerization process for a period of 8 days. The plot shows the decrease in the relative peak area of the rigid amylopectin peak observed with a T_2 of ca. 10 μs in the FID of the fully deuterated starches in D_2O. The effect of Lintnerization can therefore be seen in a direct

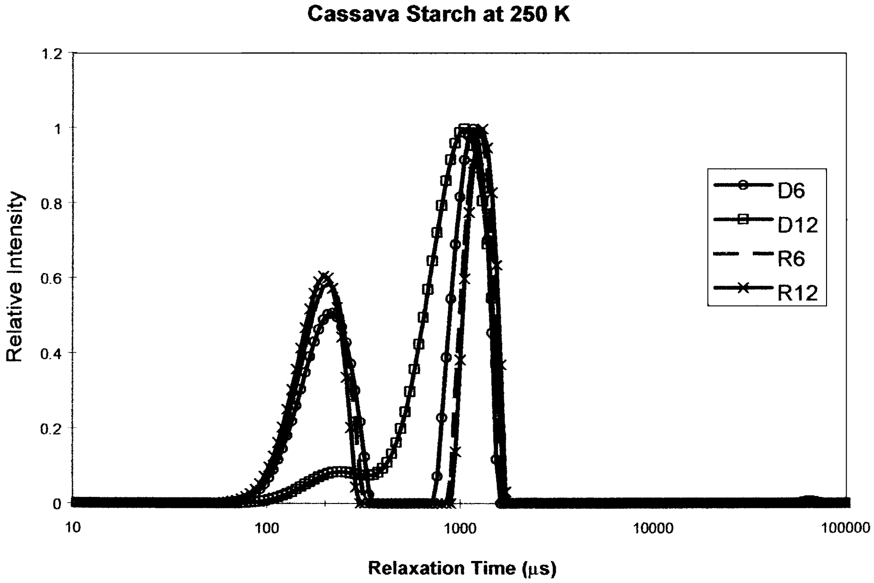

Fig. 12. Comparison of the CPMG proton relaxation spectra for cassava starches frozen in water (H_2O) at 250 K.

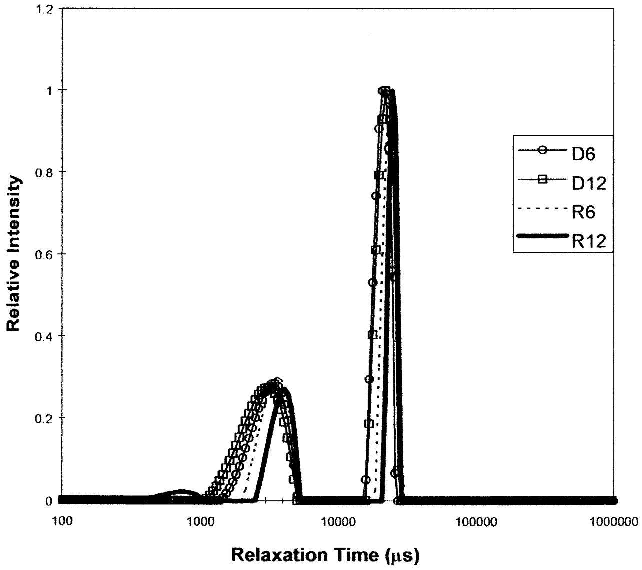

Fig. 13. Comparison of the CPMG relaxation spectra for cassava starches at 277 K in a water (H_2O)-saturated bed.

comparison of Fig. 3 (no Lintnerization) and Fig. 15. The most noteworthy effect is that the removal of amylose and amorphous amylopectin from the R6 and R12 samples by acid hydrolysis has made them much harder to gelatinize. Whereas they were both gelatinized by 353 K in Fig. 3 neither sample is fully gelatinized even at 363 K in Fig. 15. In fact both samples behave much more like the D6 and D12 starches whose behaviour is hardly affected by the 8 day Lintnerization process. These results are also consistent with the idea that the amylose in the R12 and R6 samples is more accessible to acid hydrolysis and more rapidly gelatinized than in the dry season samples.

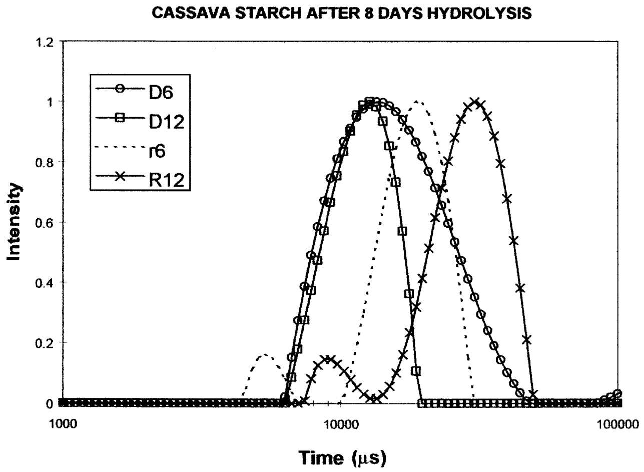

Fig. 14. The CPMG relaxation time spectrum for water (H_2O) in starches that have be subjected to 8 days acid hydrolysis and examined as a packed water-saturated bed at 298 K.

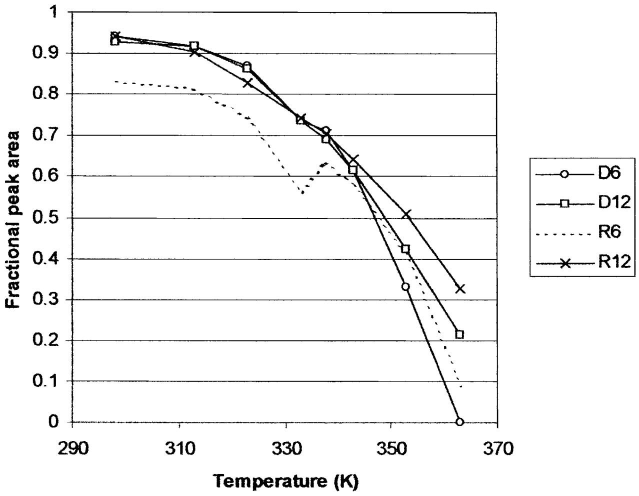

Fig. 15. The effect of 8 days Lintnerization on the gelatinization behaviour. (Cf. the results without Lintnerization in Fig. 3).

4. Discussion

The NMR relaxation spectra show that the starch chains in the rainy season samples, especially the R12 sample, are more readily plasticized and gelatinized than the dry season starch granules. This is consistent with their larger average size and therefore higher water content. The NMR data also reveals the exceptionally rapid gelatinization and acid hydrolysis of the R12 sample (see, for example, Figs. 5,6, 11 and 14). In contrast the relaxation spectra show that the amylose and amylopectin chains in the starches harvested in the dry season (D6 and D12) are more rigid and more difficult to plasticize and gelatinize than the rainy season samples (Figs. 3 and 9). It is also interesting to note that the mean amylose chain length in R12 sample is shorter than in the dry season samples and this, no doubt, facilitates their plasticization and release from the granule during heating.

All these effects are, ultimately, a consequence of the effect of water availability on the activity of the array of enzymes controlling the rates of amylose and amylopectin synthesis. Future research should therefore focus on monitoring water distribution and availability in the intact, growing cellular tissue.

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