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A study of ordered structure in acid-modified tapioca starch by ^{13}C CP/MAS solid-state NMR

Napaporn Atichokudomchai[a,b], Saiyavit Varavinit[a], Pavinee Chinachoti[c,*]

[a]*Department of Biotechnology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand*
[b]*Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011, USA*
[c]*Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA*

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Abstract

Acid modification of tapioca starch earlier reported to increase the mechanical strength of tablets. The development of ordered structure (double helices) of these starches was monitored after equilibrating at 0.90 a_w (25 °C) using ^{13}C CP/MAS NMR and X-ray diffraction. As the hydrolysis time increased, the intensity of the resonance for C1 and C4 amorphous fractions decreased while that for C1 and C4 double helix fractions increased. Relative crystallinity (%) obtained from ^{13}C CP/MAS NMR and X-ray diffraction methods both increased sharply initially and then levelled off with hydrolysis time. The initial increase in relative double helix content and crystallinity was due to a hydrolytic destruction in the amorphous domain, retrogradation of the partially hydrolyzed amylose and crystallization of free amylopectin double helices. After 192 h, these two parameters were not significantly different ($\alpha = 0.05$) indicating that the double helices that were not arranged into crystalline regions either had been hydrolyzed or crystallized.

Keywords: Acid-modified; Tapioca; Cassava; Starch; NMR; Ordered structure; Crystallinity; ^{13}C CP/MAS; X-ray diffraction

1. Introduction

Molecular order in starch granule composes of two types of helices from amylopectin side chains. Helices that are packed in regular arrays forming crystallinity, which can be measured by both X-ray diffraction and ^{13}C CP/MAS solid state NMR. Helices that are not packed in regular form or packed in the short-range distance cannot be detected by X-ray diffraction but can still be detected by ^{13}C CP/MAS solid state NMR (Cooke & Gidley, 1992). It is therefore not surprisingly that estimates of order proportion by NMR spectroscopy are considerably higher than those of obtained from X-ray diffraction.

^{13}C CP/MAS solid state NMR of C1 resonance contains information both on the crystalline nature as well as the non-crystalline (but rigid) chains. The multiplicity of the C1 resonance corresponds to the packing type of the starch granules. For A-type starch, maltotriose is the repeat unit and the twofold axis generates the double helix, thus the C1 peak in A-type starch spectra is a triplet. While for B-type starch, maltose is the repeat unit and the threefold screw axis generates the double helix, thus the C1 peak in B-type starch spectra is a doublet (Gidley & Boceik, 1985; Veregin, Fyfe, Marchessault, & Taylor, 1986).

Morrison, Tester, Gidley, and Karkalas (1993) reported the following major changes in the ^{13}C CP/MAS-NMR spectra of waxy and non-waxy barley starches following acid hydrolysis. In the C1 region (90–110 ppm) intensity was greater in the range 99–102 ppm (characteristic of double helices) and less in the range 93–99 ppm (part of the signal develop from non-ordered material). The peaks in the C2,3,4,5 region (70–79 ppm) were sharpening and the signal near 76 ppm (B-type double helices from residues of free amylose) was pronounced for the non-waxy starches. Base on these observations, the above authors postulated that lintnerization increased the double helix content relative to non-ordered conformations. The authors also

* Corresponding author. Address: Hill's Pet Nutrition, Inc., Science and Technology Center, P.O. Box 1658, Topeka, KS 66601-1658, USA. Tel.: +1-785-286-8551.
E-mail address: pavinee_chinachoti@hillspet.com (P. Chinachoti).

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showed, by ^{13}C CP/MAS NMR, that the double helix content increased due to retrograded free amylose on lintnerization of non-waxy starches but were little changed for waxy starches.

In our previous study, native and acid-modified tapioca starches with 6% HCl were compressed into tablets at various compression forces. It was found that native tapioca starch provided low crushing-strength tablets while the crushing strength of tablets preparing from acid-modified tapioca starches increased inline with the crystallinity (Atichokudomchai, Shobsngob, Chinachoti, & Varavinit, 2001). The results suggested that the acid-modified tapioca starches could potentially be used as tablet filler in direct compression process. The objective of the present work was to study the ordered structures in acid-modified tapioca starch by ^{13}C CP/MAS solid-state NMR. The double helix contents and the crystallinity of acid-modified starches would be analyzed by peak fitting procedure and compared with the crystallinity that derived from X-ray diffraction technique. The knowledge from this study would be used for elucidating the structure of acid-modified tapioca starch in its utilization as tablet filler in pharmaceutical industry.

2. Materials and method

2.1. Materials

Tapioca starch was the product of Choheng Co., Ltd (Sampran, Nakhonprathom, Thailand). The chemical compositions of the starch were 0.08, 0.19 and 0.39% (w/w) of protein, lipid and ash content, respectively (Atichokudomchai et al., 2001). Sodium hydroxide and hydrochloric acid were purchased from Merck (Germany).

2.2. Methods

2.2.1. Preparation of acid-modified tapioca starches

Four hundred gram (dry basis) of tapioca starch was hydrolyzed by suspending in 600 ml of 6% (w/v) HCl solution at 25 °C for 0, 12, 24, 48, 96, 192, 384 and 768 h without stirring. After each hydrolysis time, the suspension was neutralized with diluted NaOH solution, and washed three times with distilled water. The water was then removed by centrifugation (Sorvall RC 3B Plus, Du Pont Company, Delaware, USA) at 1000 rpm for 2 min and decanting. The acid-modified starch slurry was spray-dried with a mobile minor spray dryer (Gea-Niro, Denmark) at the inlet and outlet temperatures of 160 and 60 °C, respectively. The dried powder was sieved through 100-mesh sifter to obtain acid-modified starch powder. Spray-dried native tapioca starch was defined as a 0-h sample (control).

2.2.2. Preparation of amorphous starch

Amorphous starch was prepared by gelatinizing tapioca starch in Brabender Viscoamylograph Type E (Duisburg, Germany). A 6% (w/v) of tapioca starch suspension was heated from 25 to 95 °C at the rate of 1.5 °C/min, held at 95 °C for 15 min and then cooled to 50 °C also at the rate of 1.5 °C/min. The starch suspension was spreaded to form thin layer before drying overnight in an oven (60 °C). The amorphous starch sample (containing 10% water) was blended and sieved through a 100-mesh sifter to obtain the amorphous starch powder.

2.2.3. Water content adjustment

The water content of all samples was adjusted to 0.90 water activity (a_w) over $BaCl_2$ saturated salt solutions at 25 °C.

2.2.4. X-ray powder diffraction measurements

Monochromatic Cu-K_α radiation (wavelength = 1.542 Å) was produced by a Bruker D8 (German) X-ray powder diffractometer. The equilibrated starch powders at RH = 0.90 were exposed to the X-ray beam with the X-ray generator running at 40 KV and 30 mA. The scanning regions of the diffraction angle 2θ were 4°–30°, step interval 0.02, scan rate 2°/min. The sollet and divergence slit was 1°. The receiving slit was 1°and scattering slit was 0.15°. The measurements were made at 25 °C. Relative crystallinity (%) of the starches were measured following the method of Komiya and Nara (1986).

2.2.5. Solid state NMR experiments

The CP-MAS ^{13}C NMR spectra were recorded on a Bruker MSL 300 (Bruker Instrument, Inc., Billerica, MA) equipped with CP-MAS accessories. Dipolar decoupling was systematically used during the acquisition sequence. The samples were spun at a rate of 5 kHz at room temperature in a 7 mm rotor, the accumulation of 800 was used to obtain a satisfactory signal to noise ratio. The optimal contact time was 1 ms, spectral width 30 kHz, acquisition time 30 ms, time domain points 2 K and line broadening 10 Hz. Spectra were analyzed using, the high field resonance, adamantane (29.5 ppm) as the reference.

2.2.6. Peak fitting procedure

The CP-MAS ^{13}C NMR spectra were peak fitted by using PeakFit™ version 4 for Win 32 (Jandel Scientific Software, CA). The percentage of double helix content (ordered parts) in the starches was calculated following Bogracheva, Wang, and Hedley (2001)'s method;

The precentage of amorphous parts in native starch

$$= \frac{\text{PPA for the native starch}}{\text{PPA for amorphous starch}} \times 100$$

Double helix content (%) = 100 − amorphous part (%). When the PPA was defined as the proportion of C4 peak fitting area relative to the total area of the spectrum.

The relative crystallinity (%) was calculated according to the method described by Paris, Bizot, Emery, Buzare,

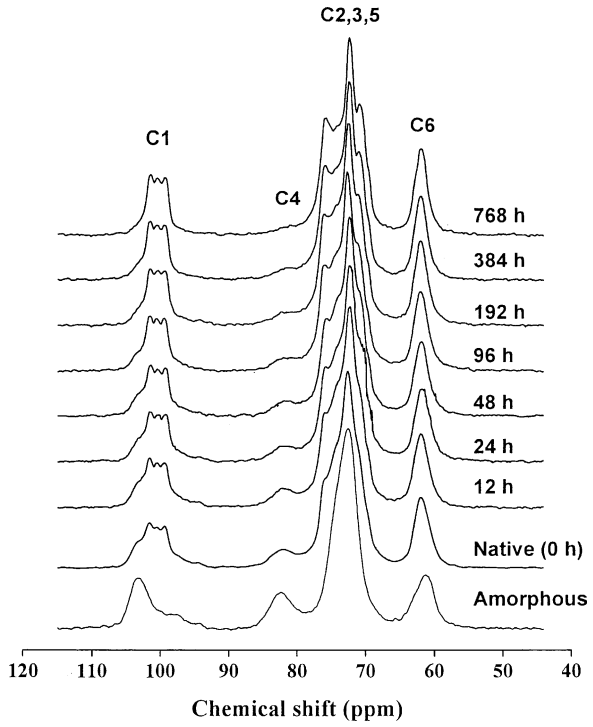

Fig. 1. ^{13}C CP/MAS NMR spectra of amorphous, native and acid-modified tapioca starches at various hydrolysis times, i.e. 12, 24, 48, 96, 192, 384, and 768 h after equilibrating at 0.90 a_w (25 °C).

and Buleon (1999). The percentage of relative crystallinity was calculated as the proportion of the fitting peak areas of the triplet relative to the total area of the C1 spectrum.

3. Results

Fig. 1 summarizes the ^{13}C CP/MAS NMR spectra of amorphous, native (control) and acid-modified tapioca starches at various hydrolysis time. ^{13}C chemical shift values for all resolved signals are given in Table 1. Assignments of the resonance were consistent with literature data (Gidley & Boceik, 1985; Veregin et al., 1986). Signals at 94–105 and 58–65 are attributed to C1 and C6 in hexapyranoses, respectively. The overlapping signal around 68–78 ppm is associated with C2, C3 and C5. The C1 resonances of both native and acid-modified tapioca starches are triplets, which is a typical A-type characteristic (Fig. 1). The two broad shoulders that appeared at 103 and 95 ppm could arise from the amorphous domains for C1 and the broad resonance at 82 ppm from amorphous domains for C4. Such assignment was based on the fact that these broad resonances are absent in the A- and B-type spherulitic crystal spectra, but present dominantly in that from amorphous samples (Gidley & Boceik; Veregin et al.)

Table 1
^{13}C CP/MAS NMR chemical shifts of amorphous, native and acid-modified tapioca starches at various hydrolysis time equilibrated at 0.90 a_w (25 °C)

Tapioca starch sample	Moisture content (%)	Chemical shifts (ppm)			
		C1	C4	C2, 3, 5	C6
Amorphous (gelatinized)	21.0±0.8	103.133	82.303	72.561	61.191
Native (control)	18.7±0.2	101.522 100.445 99.303	82.119	72.436	62.028
Acid-modified (hydrolysis time (h))					
(12)	18.6±0.4	101.461 100.391 99.315	82.191	72.397	61.989
(24)	18.7±0.6	101.533 100.452 99.340	81.879	72.450	62.020
(48)	18.9±0.2	101.484 100.498 99.345	81.202	72.453	62.002
(96)	18.8±0.2	101.490 100.525 99.433	nd	75.887 72.476	62.042
(192)	18.7±0.5	101.430 100.351 99.338	nd	75.900 72.428	62.014
(384)	18.9±0.2	101.471 100.458 99.388	nd	75.941 72.471 71.094	62.046
(768)	18.9±0.2	101.474 100.497 99.376	nd	75.990 72.477 71.033	62.089

nd, not detectable.

(Fig. 1). The intensity of C1 and C4 amorphous resonance were found to decrease with increasing hydrolysis time and almost disappeared when the hydrolysis time reached 768 h. Sharpening of acid-modified spectra was observed (Fig. 1).

The spectrum of gelatinized tapioca starch (Fig. 1) was very similar to that of other amorphous starch, e.g. gelatinized and ethanol precipitated potato starch (Gidley & Boceik, 1985) and extruded potato starch (Paris et al., 1999). This suggested that the spectrum for amorphous starch is not related to the type and method from which it was produced. The amorphous sample spectrum was subjected to Gaussian curve fitting (Fig. 2). Native and acid-modified starches fitted spectra are compared in Fig. 3. A combination (50/50) of Lorentzian and Gaussian profiles gave acceptable fitting ($r^2 \geq 0.9990$).

Fig. 4 depicts the calculated relative double helix contents (^{13}C CP/MAS NMR) and the relative crystallinity (^{13}C CP/MAS NMR and X-ray diffraction) at various hydrolysis time but same RH (90% at 25 °C). In the first 24 h of the hydrolysis, the crystallinity obtained from both techniques increased significantly in a faster rate than the double helix (Fig. 4). During 24–96 h, relative double helix content and crystallinity increased with increasing in hydrolysis time in parallel fashion. All parameters stabilized after 192 h of hydrolysis. The relative double helix content was higher than the relative crystallinity (both from ^{13}C CP/MAS NMR and X-ray diffraction) in the first stage of the hydrolysis (12–96 h). After 192 h of the hydrolysis, these two parameters were not significantly different ($\alpha = 0.05$) (Fig. 4). Crystallinity approximated by ^{13}C CP/MAS NMR was found higher than that approximated from X-ray diffraction in the first stage of the hydrolysis.

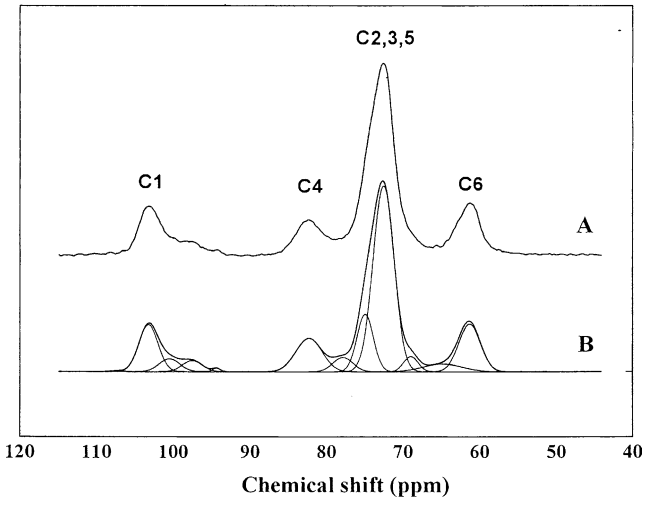

Fig. 2. (A) ^{13}C CP/MAS NMR spectrum of amorphous tapioca starch after equilibrating at 0.90 a_w (25 °C), (B) Peak fitted to the NMR average spectrum of amorphous tapioca starch. All the fitted peaks were Gaussian shape and the overall peak fitting correlation coefficient was 0.9998.

4. Discussion

It is known that water has a significant effect on molecular properties of starch. Presence or absence of residual water can lead to helical formation of the linear chains and a crystallinity increase. These lead to observable change in ^{13}C CP/MAS NMR and X-ray diffraction pattern (Bogracheva et al., 2001; Cheetam & Tao, 1998; Gidley & Boceik, 1985; Paris et al., 1999; Veregin et al., 1986). In this study, starch samples were adjusted to the same RH of 90% (21% moisture content for amorphous sample and 18.7 ± 0.2% for remaining samples). Although the amorphous sample contained higher moisture content (21%), the amorphous domains are similarly hydrated as the crystalline domains suggested by a relatively small difference in sorption behavior between native and gelatinized starches (Bizot, Buleon, Mouhous-Riou, & Multon, 1985; Paris et al.).

Acid-modified starches show triplets C1 spectra similarly to that of the native starch (Fig. 1), indicating that acid-modification did not have an effect on molecular packing of the double helices in the crystalline regions. In general, amorphous compounds give broad resonances as the distribution of local molecular environments give rise to a broad distribution of chemical shifts for each carbon. Ordered materials show narrower resonances due to more regularity of the environment (Gidley & Boceik, 1985; Veregin et al., 1986), reflecting the stricter polymer configurations in the ordered parts of the starch (Paris et al., 1999). Since the resonances that appeared at 103 and 95 ppm (left and right shoulders of C1 peak) and at 82 ppm of C4 are believed to be contributed by the amorphous components, their disappearance and the dramatic ^{13}C CP/MAS NMR spectral sharpening with the increasing in hydrolysis time (Fig. 1) suggested favorable hydrolysis of the amorphous regions in the starch granules. This is in agreement with the previous works (Gidley & Boceik; Morrison et al., 1993; Veregin et al.).

Fig. 4 shows the relative double helix content estimated from ^{13}C CP/MAS NMR comparing with their relative crystallinity obtained from both ^{13}C CP/MAS NMR and X-ray diffraction. In the first stage of the hydrolysis (12–96 h), the relative crystallinity obtained from ^{13}C CP/MAS NMR was higher than those obtained from X-ray diffraction method (possibly due to the chain regularity being affected differently as seen by the two techniques, Paris et al., 1999). Native starch contained higher double helix content than the crystallinity (Fig. 4). The result was in agreement with the general acceptance (Cooke & Gidley, 1992; Gidley & Boceik, 1985). It has been earlier reported that double helix content of a starch (^{13}C CP/MAS NMR data) increased significantly (26%) due to retrograded free amylose upon lintnerization of non-waxy starches, but very little change (2%) was observed for waxy starch (Morrison et al., 1993). In our previous study on the gelatinization transitions of these acid-modified tapioca starches

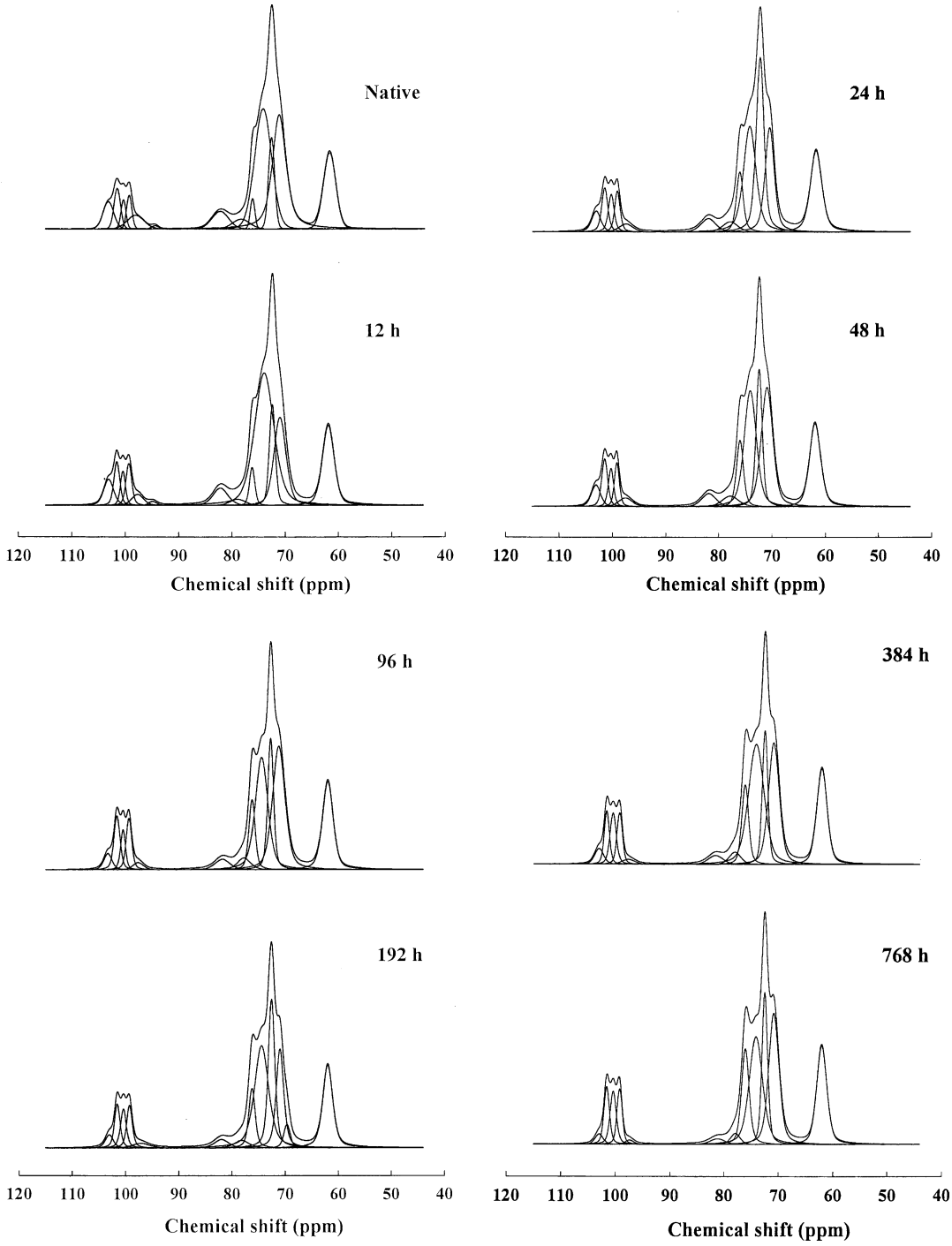

Fig. 3. Fitted peak profiles to the ^{13}C CP/MAS NMR spectra for native and acid-modified tapioca starches at various hydrolysis times, i.e. 12, 24, 48, 96, 192, 384 and 768 h. The peak fitting correlation coefficient was at least 0.9990.

(Atichokudomchai, Varavinit, & Chinachoti, 2002), the increase in the onset temperature in the first stage of the hydrolysis was due to the melting of the retrograded amylose. Therefore, the sharp initial increase in the double helix content (Fig. 4) would be partly due to the increase in double helices derived from retrograded partially hydrolyzed amylose. This was supported by the increase in the intensity of the peak around 76 ppm (Fig. 1) earlier assigned to the B-type double helices from residues of free amylose (Morrison et al.). The faster increasing rate of the crystallinity than the double helix in the first 24 h of the hydrolysis (Fig. 4) suggesting that the retrograded amylose double helices were readily arranged into crystalline regions and the hydrolysis of branch structures of amylopectin in the amorphous region might help releasing constrain of the molecules that caused by the branch linkages and allow better alignment of double helices to form crystalline structure, thus the crystallinity was increased faster than

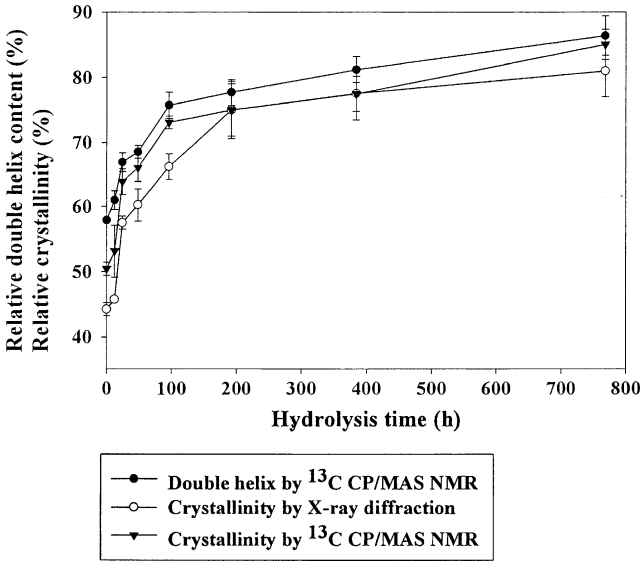

Fig. 4. Comparison of relative double helix content (%) obtained from ^{13}C CP/MAS NMR with relative crystallinity (%) obtained from ^{13}C CP/MAS NMR and X-ray diffraction methods of native and acid-modified tapioca starches at various hydrolysis times after equilibrating at 0.90 a_w (25 °C).

the double helix. During 24–96 h, the increase in double helix was in the same manner with the relative crystallinity (Fig. 4), implying that most of the crystallization of the free amylopectin double helices finished within the first 24 h while the retrograded amylose double helices were constantly arranged into crystalline regions (otherwise the double helix content would have increased in a faster rate than the relative crystallinity). The structure of retrograded amylose was proposed by Jane and Robyt (1984). They suggested that amylose retrograded forming crystalline regions that are interspersed with the amorphous regions. The amorphous regions could be further hydrolyzed by acid and leaving the crystalline regions intact (Jane & Robyt, 1984).

In the second stage of hydrolysis (192–768 h), the relative double helix content was not significantly different from the relative crystallinity (Fig. 4) indicating that most of the double helices left from the first stage of the hydrolysis were either hydrolyzed or crystallized. The results supported the previous theory (Atichokudomchai et al., 2001) that the increase in the crushing strength of the tablets preparing from these acid-modified starches was due to the increase in the crystallinity in the starch granules and that extensive hydrolysis could destroy double helices and crystalline domains. The erosion of the amorphous regions by acid hydrolysis may reduce the hindrance between double helical chains. Thus, when applying the compaction force to the starch granules, the crystalline regions could be forced to become closely packed together, so the intermolecular forces, i.e. Van de Waals force and H-bonding, increase. The hydrolysis of the amorphous regions at the branch points may also allow the amylopectin branch-chains to be more mobile, leading to a more order rearrangement within the starch granules when it is compressed. Moreover, the formation of the partially hydrolyzed retrograded amylose may also help increase the crushing strength, since it contains crystalline regions which are resistant to the acid hydrolysis (Jane & Robyt, 1984).

5. Conclusion

Relative double helix content determined by ^{13}C CP/MAS solid state NMR was increased due to a destruction in the amorphous domain following acid hydrolysis. This was accompanied by a parallel increase in relative crystallinity. The rapid increase in the relative crystallinity in the first stage of the hydrolysis could also be due to the crystallization of the retrograded amylose and free amylopectin double helices. In the second stage of the hydrolysis, the double helix content was not significantly different to the relative crystallinity determined by ^{13}C CP/MAS solid state NMR and X-ray diffraction indicating that most of the double helices left from the first stage of the hydrolysis either were hydrolyzed or crystallized. The increasing in crystallinity and more ordered structure (double helix) of acid-modified starches resulted in an increase in crushing strength of the tablets.

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