13C MAS NMR Studies of the Effects of Hydration on the Cell Walls of Potatoes and Chinese Water Chestnuts

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13C NMR with magic angle spinning (MAS) has been employed to investigate the cell walls of potatoes and Chinese water chestnuts over a range of hydration levels. Both single-pulse excitation (SPEMAS) and cross-polarization (CPMAS) experiments were carried out. Hydration led to a substantial increase in signal intensities of galactan and galacturonan in the SPEMAS spectra and a decrease in line width, implying mobilization in the backbone and side chains of pectin. In CPMAS spectra of both samples, noncellulose components showed signal loss as hydration increased. However, the signals of some galacturonan in the 3_1 helix configuration remained in the spectra even when the water content was as high as 110%. Cellulose was unaffected. It is concluded that the pectic polysaccharides experience a distribution of molecular conformations and mobility, whereas cellulose remained as typical rigid solid.

Keywords: *Plant cell walls; hydration; MAS NMR; potato; Chinese water chestnut*

INTRODUCTION

Plant cell walls are of vital importance to the activity of cells by providing the rigidity to keep cells intact and the mobility to enable cells to communicate and reproduce (Brett et al., 1990). Cell walls are also of significance to the textures of foods with a plant origin (Muramatsu et al., 1996; Waldron et al., 1997). However, an understanding of the molecular structure and dynamics and their relationships to the mechanical, biochemical, and biological properties of cell walls remains far from complete.

It is well-known that the chemical composition of cell walls varies from source to source. Nevertheless, they are mostly composed of cellulose, pectin, hemicellulose, proteins, and polyphenols (Brett et al., 1990). Cellulose is an unbranched β-1,4-glucan present mostly in the form of crystalline microfibrils. Pectin generally consists of a group of polysaccharides rich in galacturonic acid, rhamnose, arabinose, and galactose. These polysaccharides are present in the form of galactan, galacturonan, rhamnogalacturonan, arabinogalactan, and arabinan (Brett et al., 1990). Hemicellulose is often used as a convenient term for xylan, glucomannans, and xyloglucans and is thought to interact with cellulose strongly through a network of hydrogen bonding (Brett et al., 1990). Cellulose microfibrils form the framework, which hemicellulose and pectin hold together by formation of a matrix (Brett et al., 1990). The microfibrils are responsible for strength, whereas the matrix components are responsible for the charge, porosity, and hydrophobic/hydrophilic characteristics of cell walls (Brett et al., 1990).

Spectroscopic methods have proved to be very powerful in polymer studies (McBrierty et al., 1993) and in cell wall studies (Mackay et al., 1982, 1988; Taylor et al., 1990; Tang et al., 1998a–d; Foster et al., 1996; Jarvis et al., 1995, 1996; Ha et al., 1996; Newman et al., 1994, 1996; Koh et al., 1996, 1997; Smith et al., 1998a,b). Solid-state NMR has been applied with some success. Some 1H NMR relaxation studies (Mackay et al., 1982, 1988; Taylor et al., 1990) have yielded some tentative assignments of components having different relaxation behaviors. More recently, we have carried out a systematic investigation on the molecular dynamics of the cell walls (Tang et al., 1998c,d) and model systems (Tang et al., 1998a,b) as a function of temperature and hydration levels using 1H relaxation measurements. Some effort has been made to relate the proton relaxation properties of cell wall components with their structural details (Ha et al., 1996; Jarvis et al., 1996). Solid state 13C NMR studies of cell walls have also led to spectral assignments (Foster et al., 1996; Jarvis et al., 1995, 1996; Ha et al., 1996; Newman et al., 1994, 1996; Koh et al., 1996, 1997; Smith et al., 1998a,b). However, most research reported so far has used cell wall materials in poorly defined conditions of temperature and hydration. Substantial effects of hydration and temperature on cell wall relaxation dynamics have been shown by our previous work (Tang et al., 1998c). However, there is no published report, as far as we know, dealing systematically with the effects of hydration on cross-polarization magic angle spinning (CPMAS) spectra of cell walls.

EXPERIMENTAL PROCEDURES

Materials. α-Cellulose, polygalacturonic acid from orange, and pectin (degree of esterification = 93%) from citrus fruits were purchased from Sigma and used without further purification. They are referred to as cellulose, PGA, and P93, respectively. Cell wall materials of potatoes were obtained from two preparation methods described previously (Ryden et al., 1990; Parker et al., 1995; Tang et al., 1998c) and referred to as PA (Ryden et al., 1990) and PB (Parker et al., 1995), respectively. In the PA preparation, which used a DMSO extraction step, previous work (Tang et al., 1998c) has sug-

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gested that there is residual contamination with DMSO. However, this sample has been included here to compare with the sample PB. Cell walls of Chinese water chestnut were prepared using the Waldron method (Parker et al., 1995) and are referred to as CWC.

Cell walls were all freeze-dried and then dried in a vacuum oven over P_2O_5, at 40 °C, for at least 24 h. Samples were used only after they had been stored over P_2O_5 in a desiccator under vacuum for at least 12 h. Rehydration was carried out in a sealed jar over a saturated solution of suitable salts. Hydration levels were defined as grams of water per 100 g of dry matter and determined gravimetrically as the difference between the hydrated sample and the dry one (water content of the dry cell walls is ~2% w/w as measured by using the Karl–Fisher titration method).

13C MAS Experiments. All MAS experiments were carried out at 303 K on a Bruker MSL-300 spectrometer operating at 300.13 and 75.46 MHz for ^1H and ^{13}C, respectively. A Bruker double-bearing MAS (DBMAS) probee head and a 7 mm zirconia rotor were employed with typical sample spinning rate of ~3.5–5 kHz.

For the single-pulse excitation with MAS (SPEMAS) spectra, a single 90° pulse of 4 μs was used to excite ^{13}C magnetization and proton-decoupled (67 kHz) during acquisition time. Recycle delay was 0.5–1 s unless stated otherwise. CPMAS spectra were recorded with a single contact time of 1–1.2 ms following a 90° proton pulse of 4 μs. Hartman–Hahn matching was set up using adamantane (16K data, contact time = ~8 ms) and verified with polycrystalline glycine (2K data, contact time = 1.2 ms). Proton decoupling power during acquisition time was maintained from the spin-locking field. The strength of radio frequency power in both proton and carbon channels was optimized by careful tuning of the probe head for both frequencies. Although perfect Hartman–Hahn matching was difficult to verify for each individual sample because of the number of transients required to obtain adequate signal-to-noise ratios, there was no obvious problem of matching loss for any sample we studied here. Glycine was used as an external chemical shift reference (176.03 ppm for the carbonyl peak). FIDs obtained from these experiments were processed with an Aspect 3000 computer or transferred onto a Silicon Graphics workstation and processed using FELIX 2.30 (Biosym).

RESULTS AND DISCUSSION

Assignment of ^{13}C NMR Spectra. Assignments of ^{13}C resonances of pectin and cellulose alone and in cell walls are well-known. However, we recorded spectra of plant cell walls, PGA, P93 (see Experimental Procedures) and α-cellulose (Figures 1 and 2) because in CPMAS experiments exact reproduction of chemical shifts from experiment to experiment can be problematic, especially when an external reference is used. We therefore have recorded chemical shifts under our experimental conditions. The resonances are given in Table 1 and assigned according to both literature work and our model systems.

The CPMAS spectrum (Figure 1i) of PGA agrees with the previously published results (Jarvis et al., 1995), whereas the spectra of highly methylated (93% DM) P93 were not found. Chemical analysis of P93 showed that it contained small amounts of Rha, Xyl/Ara, and Gal but mostly galacturonic acid (~95% mol/mol, GalA), in which 93% of the carbonyl groups were methylated. P93 showed peaks at 170, 106, 101, 96, 80, 75, 72, 69, and 53 ppm (Figure 1ii). The peaks at 170 and 53 ppm were assigned to methylated carbonyl groups and methoxyl groups, respectively (Jarvis et al., 1995). Peak p at 106 ppm was assigned to Ara$f$ moieties (Joseleau et al., 1983; Pressey et al., 1984; Ryden et al., 1989). Two C-1 peaks are observed, and C-4 appears as two groups of
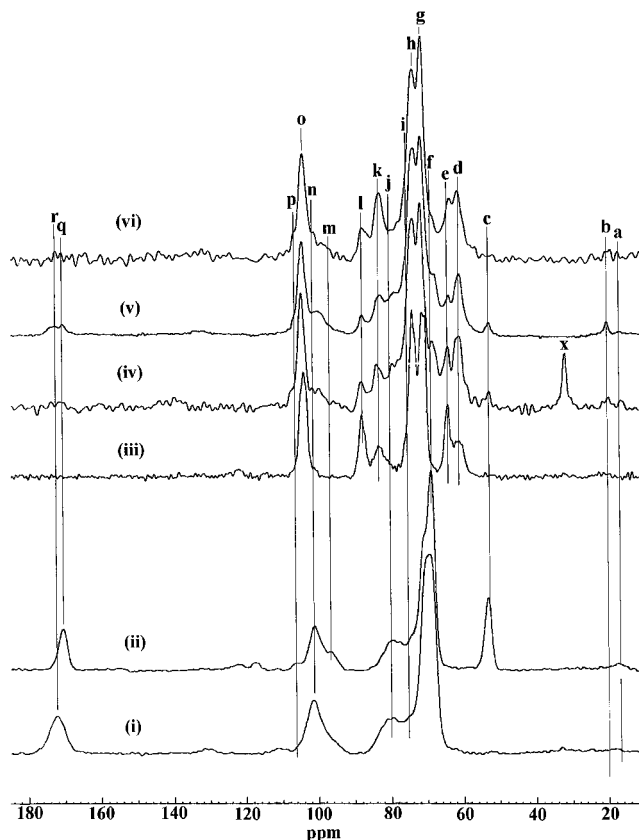
Figure 1. ^{13}C CPMAS spectra of (i) PGA (10%), (ii) P93 (16%), (iii) α-cellulose (28%), (iv) PA (40%), (v) PB (61%), and (vi) CWC (44%). Percentages in parentheses are water contents.

broad peaks. The high-frequncy peaks were assigned to the 3-fold (3$_1$) helix and the low frequency peaks to the 2-fold helix (2$_1$) (Jarvis et al., 1995). The relative intensities of the high-frequncy signals indicate that the 3-fold helix is the major conformation. However, the broadness of these peaks suggests some degree of distortion. Cellulose showed a group of peaks at ~105 ppm for C-1 and two at 88 and 83 ppm for C-4 and two at 64 and 61 ppm for C-6 (Figure 1iii). C-2,3,5 resonances were centered at 72 and 74 ppm as two groups of peaks with nearly equal intensity (Dudley et al., 1983; Horii et al., 1983, 1984). For C-4 and C-6, the high-frequency resonances were assigned to the crystalline region and the other peak to the noncrystalline form (Atalla et al., 1984; Vanderhart et al., 1984). Higher signal intensities were evident for crystalline cellulose than the noncrystalline material.

SPEMAS spectra are shown in Figure 2. P93 showed nearly all of the same peaks as in the CPMAS spectrum (Figure 1i). However, cellulose had a rather different spectrum. The C-1, C-6, and C-4 resonances are dominated by the noncrystalline signals (Figure 2ii). The pattern formed by the C-2,3,5 resonances is also different from that in the CPMAS spectrum, probably for the same reason. In the cell wall spectra (Figure 2iii,iv,v), almost all of the resonances were from pectin and noncrystalline cellulose, among which C-1, C-4, and C-6 signals are most obvious. However, signals of Gal in the form of galactan often appear at the same frequencies as glucan signals; there may be overlaps between these two polysaccharides. PA containing 40% water showed slightly better resolution than PB, which contains 61% water. This point will be dealt with this later. Peak p in the CWC spectrum is assigned to either Ara$f$ (Jose-
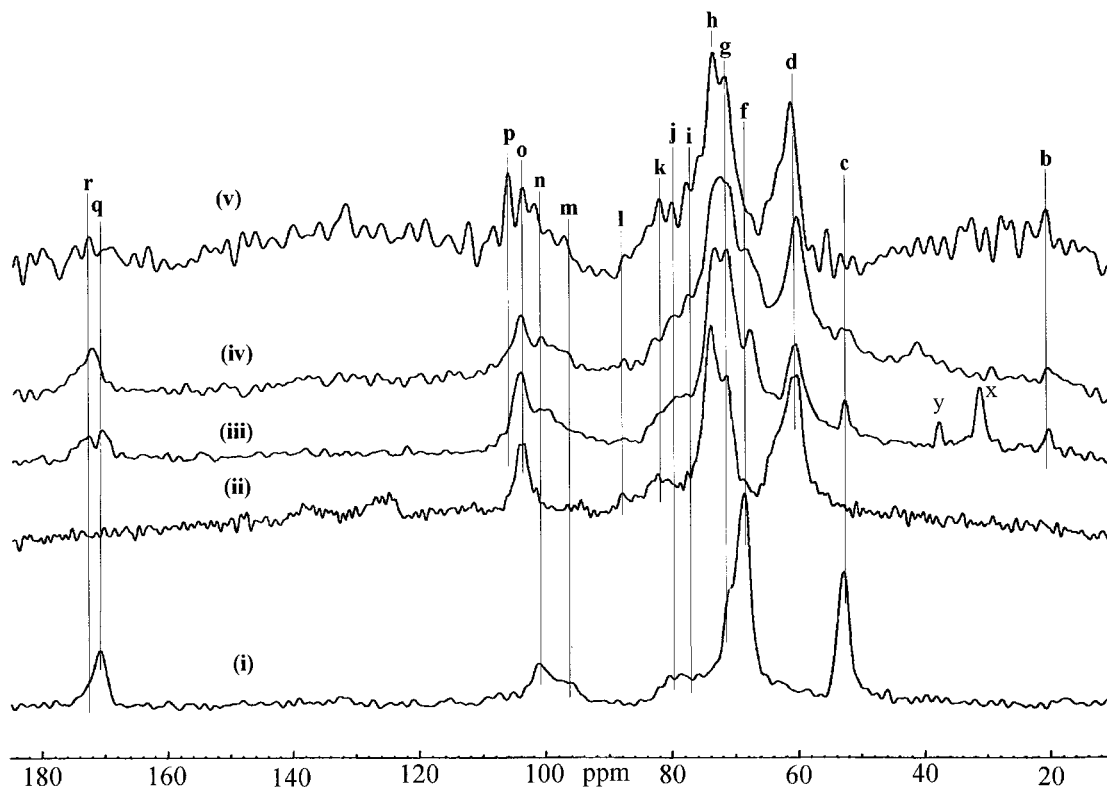
Figure 2. ^{13}C SPEMAS spectra of (i) P93 (16% H_2O), (ii) α-cellulose (28%), (iii) PA (40%), (iv) PB (61%), and (v) CWC (44%). Percentages in parentheses are water contents.
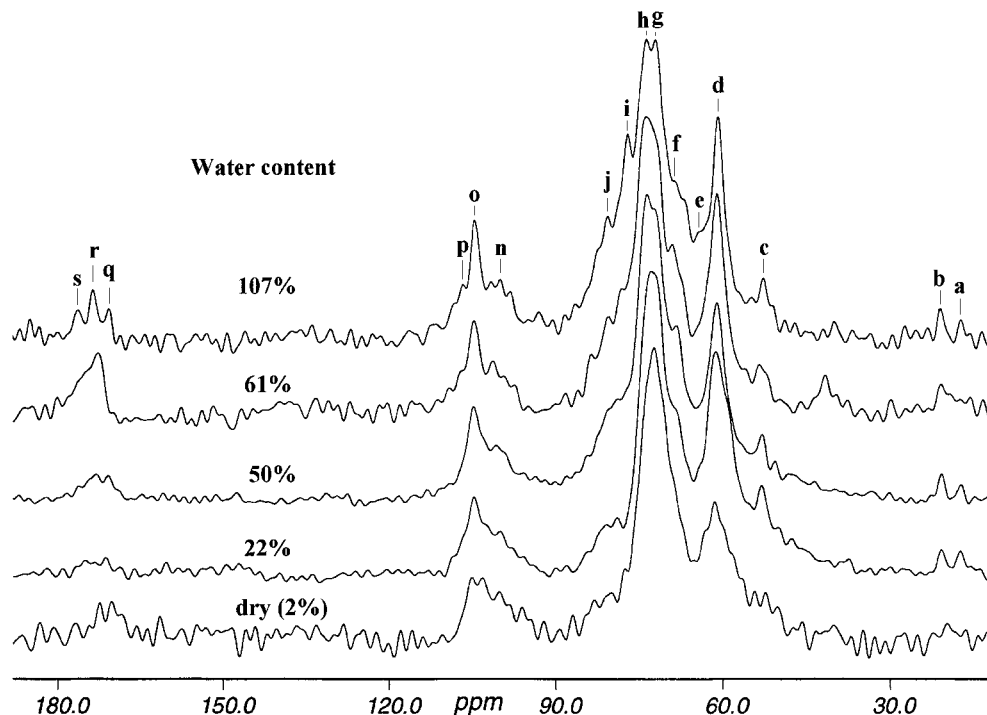
Table 1. Assignments of ^{13}C Resonances

peak	chemical shift (ppm)	assignment
a	18	C-6 (CH$_3$−) of Rha, Fuc
b	21	CH$_3$ of acetyl groups
c	53	CH$_3$O− of methyl esters
d	61	C-6, cellulose (noncrystalline), galactan
e	64	C-6, cellulose (crystalline), galactan
f	69	C-2,3,5, galacturonan (pectin)
g	72	C-2,3,5, galacturonan (pectin); cellulose
h	74	C-2,3,5, cellulose
i	75	C-4 galacturonan (pectin) in 2$_1$ helix
j	80	C-4 galacturonan (pectin) in 3$_1$ helix
k	83	C-4 cellulose (noncrystalline)
l	88	C-4 cellulose (crystalline)
m	96	C-1 galacturonan (pectin)
n	101	C-1 galacturonan (pectin) in 3$_1$ helix
o	104	C-1 cellulose, galactan
p	106	C-1 Xyl, Ara
q	170	C=O, esterified galacturonan (pectin)
r	172	C=O, protonated galacturonan (pectin)
s	176	C=O, ionic form of galacturonan (pectin)
x	32	surfactants
y	39	dimethyl sulfoxide

leau et al., 1983; Pressey et al., 1984; Ryden et al., 1989) or Xyl moieties (Matulewicz et al., 1992; Yamagaki et al., 1997) because these sugar moieties are much more abundant than in potato samples (Ara = 12.4% and Xyl = 27.1% in CWC but Ara = 6.1%, Xyl < 2% in potato samples).

Hydration Effects on Plant Cell Walls. Two types of experiments may be used to discriminate between carbon nuclei on the basis of their ability to cross-polarize and their spin−lattice relaxation behavior. In the SPEMAS experiment all carbons are excited by a 90° pulse; in principle, therefore, all carbons are observable. However, because ^{13}C relaxation times in solids are very long, a choice of a short recycle delay ensures that only signals from carbons with short T_1 are

observed. Short T_1 is often simplistically regarded as being diagnostic for mobile carbon atoms. This interpretation, while often true, should be approached with some care. The significance of short T_1 for ^{13}C is that the product of the relevant local dipolar field and the spectral densities at the carbon Larmor frequency and the sum and difference of the ^{13}C Larmor frequency and those of the neighboring dipoles are sufficiently large to cause efficient relaxation. In the case of, for example, a methyl group, this does not imply rapid isotropic tumbling but merely that rotation about one axis is rapid. In the case of CWC we have shown (Tang et al., 1998d) significant levels of free radicals to be present in the dry material. Because unpaired electron spins are a potent source of relaxation, short ^{13}C T_1 values may be observed even when the spectral densities at the appropriate frequencies are quite low. On the other hand, the observation of a CPMAS signal may be interpreted as being representative of the existence of a static local dipolar field. This means that at least some motion is in the rigid lattice regime, although as in the case of methyl groups this does not preclude highly anisotropic motion involving some motions in the rigid lattice and motional narrowing regimes.

The effects of hydration on signal line width will depend on the effects of hydration on the NMR experiment itself and the effects on the samples. Hydration may result in excess absorption of radio frequency energy, which would affect Hartmann−Hahn matching and reduce dipolar decoupling power. If these effects occur, they would produce reduced signal intensity and line broadening in the CPMAS experiments and line broadening alone in the SPEMAS experiments. Effects on the sample are likely to be mainly enhanced mobility of the molecules. This will diminish local dipolar interactions and decrease spin−lattice relaxation times.

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Figure 3. ¹³C SPEMAS NMR spectra of PB as a function of water content.

Increased motion is also likely to reduce residual chemical shift anisotropy and result in the relaxation of "frozen in" structures (Belton et al., 1993) to lower energy structures. Generally this will result in a smaller distribution of chemical shifts. Hydration is therefore likely to reduce the line width in both CP and SPEMAS spectra and result in enhanced SPEMAS signals. The observations in this paper appear to suggest that hydration effects on the sample were more important than on the NMR experiment and that matching and decoupling were not affected.

PB. SPEMAS spectra of potato cell walls at different hydration levels are shown in Figure 3. It is apparent that considerable signal intensity can be seen even when the material was very dry, corresponding to mostly pectin and probably some noncrystalline cellulose, as indicated by the C-6 signal at 61 ppm. Because this material has a very low free radical density (Tang et al., 1998d), the pectin likely has some mobility that effectively relaxes the carbons.

Hydration leads to increased spectral resolution and increased signal intensities of pectin. When the water content is 22%, signal sharpening can be seen for methyl groups (a−c), C-4 of galacturonan (i), and C-1 of noncrystalline cellulose (o). Further increase of hydration level (to 50% water) leads to further increase of spectral resolution. Carbonyl signals can be detected and a pectin resonance (n) is also distinguishable from that of cellulose (o). When the water content is increased to 61%, a small peak (p) at 106 ppm becomes detectable, corresponding to C-1 of Ara*f* (Joseleau et al., 1983; Pressey et al., 1984; Ryden et al., 1989) or Xyl in β-1,3-xylan (Matulewicz et al., 1992; Yamagaki et al., 1997), implying mobilization of arabinan and possibly xylan. Further hydration to 107% water led to further increase in resolution. Carbonyl signals were resolved into three peaks (q−s) at 176, 172 and 170 ppm, corresponding to ionized, esterified and non-ionized forms of pectin respectively (Jarvis et al., 1995). The signal of Rha/Fuc methyl groups (a) was small as expected from their low

concentration and above hydration levels of 22% no change in the signals intensity was observed, suggesting that the Rha/Fuc methyl groups were not affected by water content.

Figure 4 shows the CPMAS spectra of potato cell walls at various hydration levels. There were no significant changes in relative signal strength with contact time. Both cellulose and pectin signals are present at all the hydration levels. Nevertheless the spectra, especially the intensities of pectin signals, were critically dependent on the hydration levels. When dry, cell walls showed signals for both types of methyl groups (b, c) and galacturonan (f,g,i,j,n,q,r). As the water content increases, so does the loss of signal intensity of the peaks corresponding to the signals of galacturonan especially "j" and "n". There was a noticeable reduction of the peaks e and f, which are probably signals of Gal in galactan and GalA in galacturonan. This is consistent with the notion that increased water content increases pectin mobility such that there can no longer be efficient cross-polarization. Cellulose signals can be easily identified by the diagnostic resonances k and l at 88 and 83 ppm. Addition of water improves the spectral resolution. Cellulose signals are probably sharpened because of the disappearance of the broader pectin signals close to them whereas the narrowing of pectin signals may be due to a decreased distribution of conformations with sufficient static dipolar interactions to give CPMAS spectra. Even when the water content is as high as 107%, galacturonan signals were still observable at 170−175, 100, about 80, 53 and 21 ppm. Signals at about 100 and 80 ppm arise from 3₁ helices (Jarvis et al., 1995) and those at 53 and 21 are methyl groups. This may imply that hydration does not cause transitions in the 3₁ helix.

When the water content was as low as 22%, the peak "a" at 18 ppm was not observable. This is consistent with its observation in SPEMAS experiments, A simple anisotropic rotation of methyl groups would be insufficient to suppress static dipolar interactions and there-

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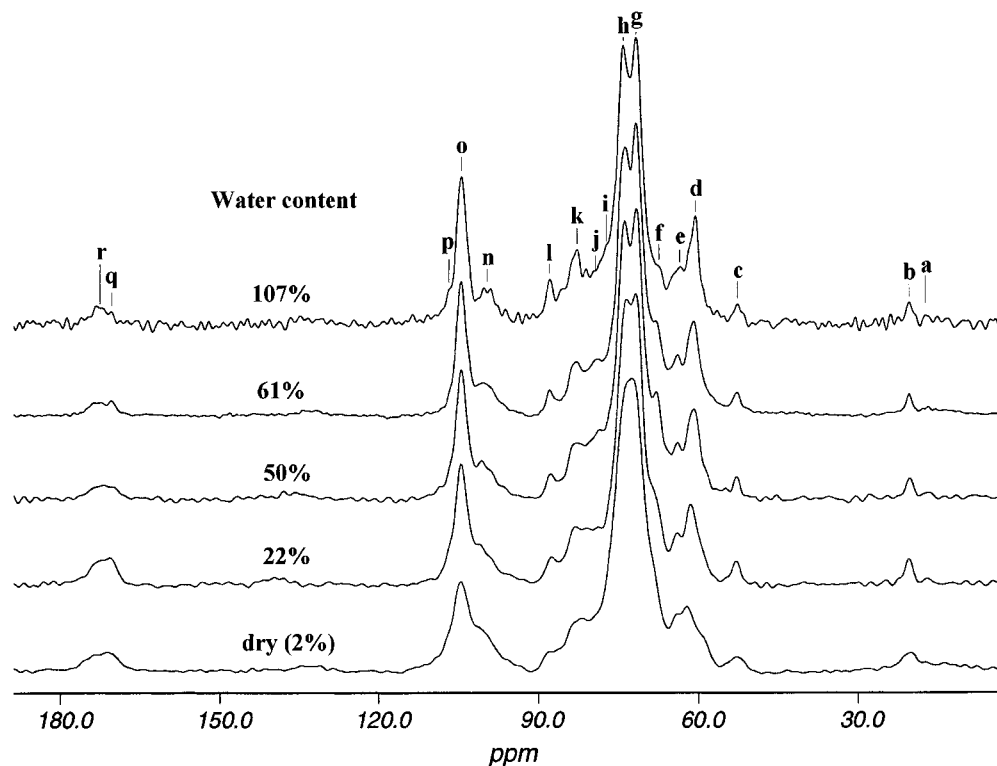
Figure 4. ^{13}C CPMAS NMR spectra of PB as a function of water content.
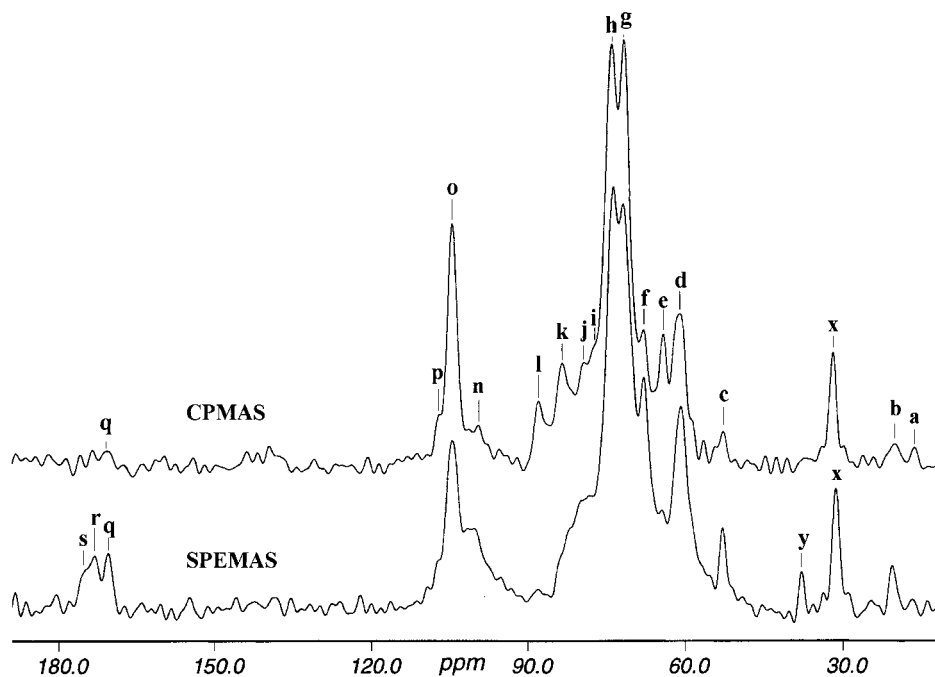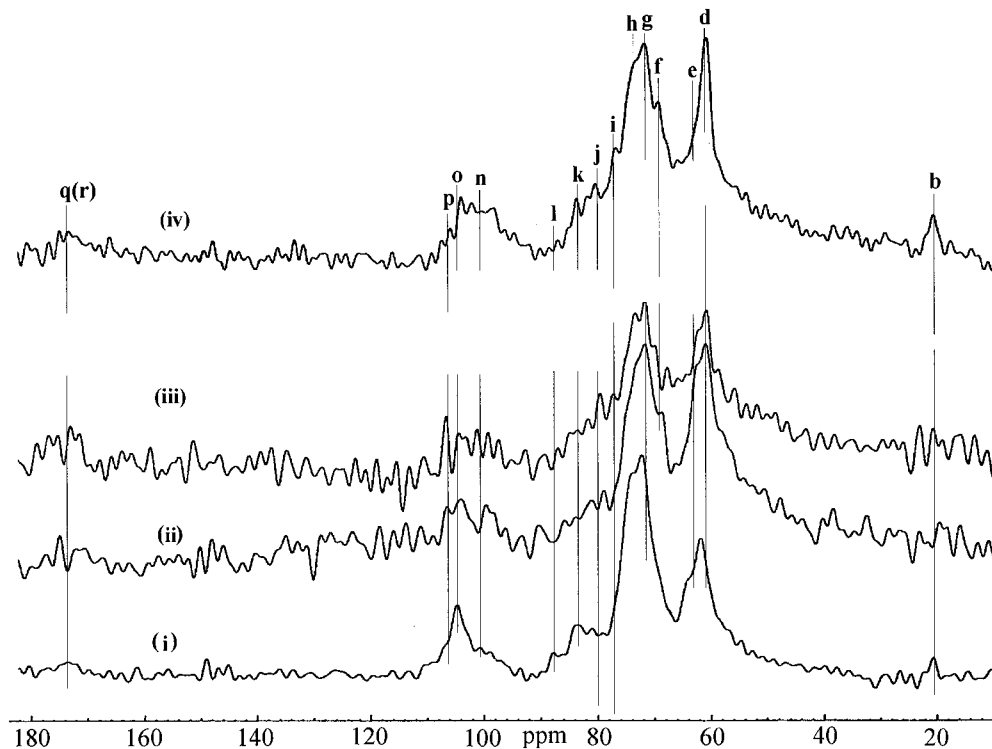

Figure 5. ^{13}C MAS NMR spectra of PA with 40% H_2O.

fore the implication is that Rha and Fuc are moving as a whole unit. Signal "p" is resolved when the water content was 107%, it may be assigned to Ara/Xyl, corresponding to arabinan (Joseleau et al., 1983; Pressey et al., 1984; Ryden et al., 1989) or xylan (Matulewicz et al., 1992; Yamagaki et al., 1997).

On the whole hydration effects on the dynamics of pectic polysaccharides much more than cellulose, this is consistent with the observations of proton relaxation (Tang et al., 1998c).

PA. Results from proton relaxation measurements indicated that sample PA was contaminated with DMSO

(Tang et al., 1998c). Comparison of spectra of PA (Figure 5) and PB (Figures 3−4) showed that while all the signals present in PB were present in PA, close inspection revealed several differences: the SPEMAS spectrum showed two very prominent signals "x" and "y" at 32 and 39 ppm which were not in the spectra of PB. They are probably signals of surfactants and dimethylsufoxide (DMSO), which were used in the process of cell wall extraction. The CPMAS spectrum of PA containing 40% water showed much less signal intensity for galacturonan at 170−175, 101, 80, 75 and 53 ppm compared with that of PB even at 60% water. This

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Figure 6. 13C SPEMAS NMR spectra of CWC as a function of water content: (i) dry (2%), recycle delay = 12 s; (ii) same as (i) but recycle delay = 1.1 s; (iii) 44%; (iv) 113%.
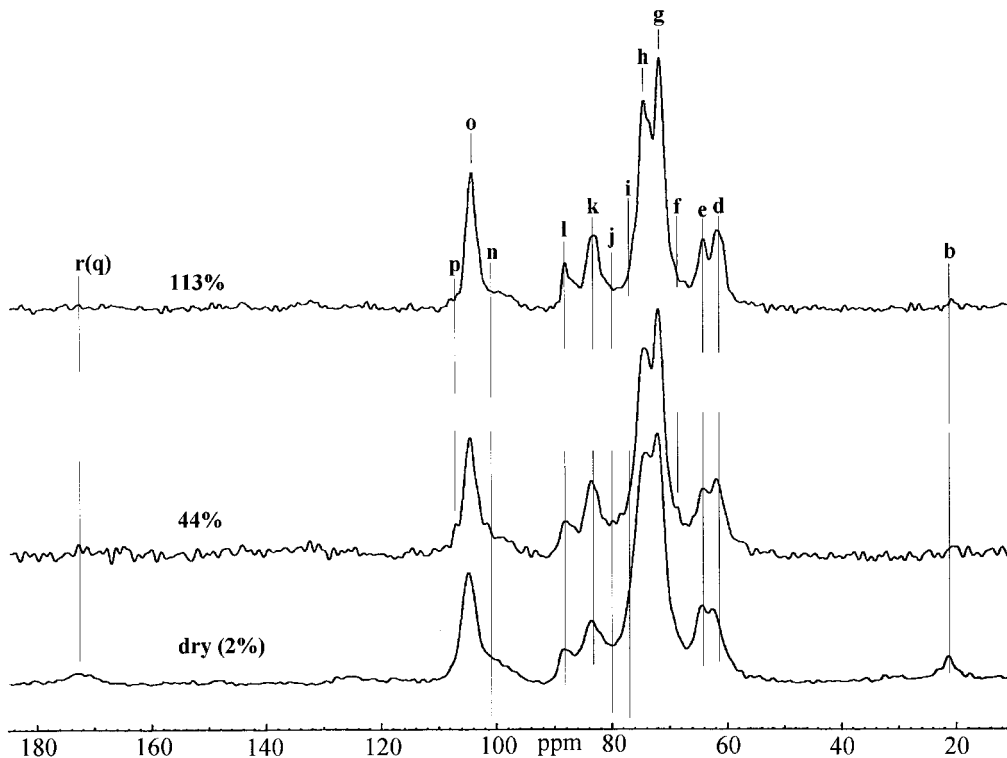
Figure 7. 13C CPMAS NMR spectra of CWC as a function of water content.

implies that galacturonan in this sample is in a more mobile state than that of PB at the same water content. This also serves as supportive evidence for the hypothesis that DMSO affects the motional properties of some pectin (Tang et al., 1998c). However, the relative intensity of peak "e" to peak "d" is much higher in PA than in PB. This probably implies that behavior of Gal moieties differs in PA and PB. Furthermore, CPMAS of PA showed much weaker carbonyl signals compared

with PB, they were resolved into three peaks in SPE-MAS which was comparable with PB containing more than 60% water. It is clear therefore that great care must be taken in the preparation of cell wall materials as both molecular dynamics and spectra are affected.

CWC. Texture of the cooked vegetables differ considerably according to their sources (Muramatsu et al., 1996; Ng et al., 1997; Parker et al., 1995; Waldron et al., 1997). Potatoes turn to be soft on cooking whereas

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Chinese water chestnuts remain crunchy (Ng et al., 1997; Parker et al., 1995). To use as a comparison with PB, cell walls of Chinese water chestnuts (CWC) were also subject to ^{13}C MAS studies.

Dry CWC showed significant signal intensity in its SPEMAS spectrum (Figure 6-ii) even when recycle delay was 1.1s. In contrast to the similar spectrum for PB, the ratio of signal intensities at 60–64 ppm (d, e) to those of g, h (70–80 ppm) were higher. The SPEMAS spectrum (Figure 6-i) with recycle delay of 12 s differs considerably in that the overall contribution of cellulose to the signal was much greater than in the short delay experiment. Some care is needed in the interpretation of these data as the dry material has been shown to contain free radicals which may affect the relaxation of some carbons more than the others. Hydration led to an increase of the relative intensities of peaks f, n, o, p assigned to pectin signals. However, close inspection revealed that the intensity of C-1 (90–110 ppm) is low relative to carbon signals at 61–67 and 70–80.

CPMAS spectra (Figure 7) of CWC showed a clear domination of cellulose signals. This is consistent with the fact that CWC contains higher content of Glc (44%) than PB. The dry sample still showed some signals (b, n, f) corresponding to galacturonan. Hydration to 44% eliminated these pectin signals, but the peak p, corresponding to Xyl/Ara$f$ (Joseleau et al., 1983; Pressey et al., 1984; Ryden et al., 1989) appeared. Further increases of the hydration levels did not change the spectra except that Xyl/Ara$f$ signals appeared to become less intense. This indicates that the pectin has a reduction in static dipolar second moment and acquires a short T_1 on hydration.

Generally, the two samples CWC and PB showed contrasting behaviors. CWC was rich in cellulose and poor in pectin, but apparently all of the pectin was mobilized on hydration. In contrast, the pectin-rich PB showed two populations of pectin, one of which was mobilized on hydration and the other, tentatively assigned to the 3_1 helix form (Jarvis et al., 1995), did not change on hydration. In contrast to the pectin results, cellulose in both samples showed little change on hydration. The results reported have shown that the state of hydration and preparative method have considerable effects on the observed spectra. It is important in the reporting of NMR results to specify the state of hydration and preparation method of the sample.

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