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ARTICLE *in* BIOMACROMOLECULES · APRIL 2011

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Residue Specific Hydration of Primary Cell Wall Potato Pectin Identified by Solid-State ^{13}C Single-Pulse MAS and CP/MAS NMR Spectroscopy

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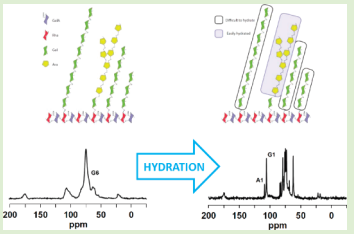
ABSTRACT: Hydration of rhamnogalacturonan-I (RG-I) derived from potato cell wall was analyzed by ^{13}C single-pulse (SP) magic-angle-spinning (MAS) and ^{13}C cross-polarization (CP) MAS nuclear magnetic resonance (NMR) and supported by ^{2}H SP/MAS NMR experiments. The study shows that the arabinan side chains hydrate more readily than the galactan side chains and suggests that the overall hydration properties can be controlled by modifying the ratio of these side chains. Enzymatic modification of native (NA) RG-I provided samples with reduced content of arabinan (sample DA), galactan (sample DG), or both side chains (sample DB). Results of these samples suggested that hydration properties were determined by the length and character of the side chains. NA and DA exhibited similar hydration characteristics, whereas DG and DB were difficult to hydrate because of the less hydrophilic properties of the rhamnose-galacturonic acid (Rha-GalA) backbone in RG-I. Potential food ingredient uses of RG-I by tailoring of its structure are discussed.



INTRODUCTION

Cell walls derived from various plants have attracted much attention during the past decades because of the importance of their functional to plant development and their industrial applications. The plant cell wall consists of a mixture of polysaccharides including cellulose, hemicellulose, and pectins.[1] The higher-level organization of these polymers into a composite with cellulose microfibrils as the main load-bearing structures embedded in a matrix of complex polysaccharides is discussed in the reviews by McCann and Knox[2] and Scheller and Ulvskov.[3] However, the major component of the cell wall matrix is water, which, in particular, interacts with the very hydrophilic polysaccharides of the pectin family (comprising, e.g., homogalacturonan, rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II)). It is the interaction with water that plasticizes the cell wall and provides viscoelastic properties.[4] It is also well-established that these properties are important to cell growth and to other aspects of plant cell function that depend on cell wall behavior.[5,6] Studies of the biomechanical properties of potato tuber tissue in which galactan or arabinan side chains of RG-I had been truncated by transgenic approaches led Ulvskov et al.[7] to propose that the RG-I structure influenced apoplastic water status and wall rheology, whereas Evered et al.[8] directly demonstrated that the hydration state influences primary walls' mechanical properties. Tang et al.[9] found that hydration greatly increased the mobility of pectic components of potato and water chestnut cell walls, and the studies of Moore et al.[10] pointed specifically to arabinan side chains of RG-I as being important to the mobility or plasticizing of pectins during water stress. The physiochemical properties of the cell wall thus depend on the polysaccharide composition, the monomer composition of the individual polysaccharides, and, for the pectins, in particular, the interaction with water molecules in the apoplastic space. These interactions may be studied by two complementary approaches: with the polysaccharides in situ, that is, in the intact wall or with the isolated polysaccharides in vitro. Hydration is not only of biological significance but is also relevant to food ingredient design. Polysaccharides are widely used as food ingredients, where they interact with water to impart the desired structure and mouthfeel. Previously, intact cell wall from onion, tomato fruit, pea stem, tobacco, potato, water chestnut, citrus, lupin, and flax were analyzed by solid-state ^{13}C NMR spectroscopy.[9,11,12] By these studies, various components in the plant cell wall were identified and spectrally assigned, and differences between cell walls from various botanical origins qwere assessed. Furthermore the mobility of arabinan and galactan side chains were compared. In general, the arabinan side chains were more mobile, but for potato cell walls, no clear distinction between the mobility of the arabinan and galactan side chains was obtained.[12] In the present work, the detailed hydration of the potato cell wall pectin, rhamnogalactorunan-I (RG-I), was investigated by ^{13}C solid-state magic-angle-spinning (MAS) NMR with and without cross-polarization (CP) of the

Received: February 10, 2011
Revised: March 22, 2011
Published: April 04, 2011

Table 1. Monosaccharide Composition (mol %) of Native Potato RG-I and Enzymatically Modified Potato RG I

monosaccharide	native (NA)	dearabinated (DA)	degalactanated (DG)	debranched (DB)
fucose	2.1 ± 0.1	2.1 ± 0.3	0.9 ± 0.1	1.3 ± 0.1
rhamnose	7.6 ± 0.3	7.7 ± 2.0	20.1 ± 2.6	23.7 ± 1.3
arabinose	12.4 ± 0.8	4.4 ± 0.1	31.9 ± 2.9	11.9 ± 3.1
galactose	69.6 ± 3.0	78.9 ± 2.1	21.4 ± 6.5	36.3 ± 3.8
glucose	n.d.	n.d.	n.d.	n.d.
xylose	0.1 ± 0.1	0.2 ± 0.2	0.6 ± 0.3	1.2 ± 0.5
GalA	8.3 ± 2.1	6.6 ± 1.7	25.0 ± 2.6	25.6 ± 3.6

isolated polysaccharides. In ^{13}C CP/MAS experiments, the resonances from immobile carbons are enhanced, whereas all carbons are detected quantitatively by the ^{13}C single-pulse (SP) MAS experiments. Using this approach, differences in mobility upon hydration were monitored for the various carbohydrate monomer units. To elucidate further the function of the arabinan and galactan side chains regarding hydration, we subjected a range of different side-chain-modified RG-Is to similar analysis.

MATERIALS AND METHODS

Sample Preparation. Intact RG-I was extracted from potato (*Solanum tuberosum* L. cv. Oleva) pulp by a large scale extraction procedure using purified enzymes, as described by Byg et al.[13] The extraction involved enzymatic removal of starch by subsequent solubilization of RG-I using a pure endopolygalacturonase. The solubilized RG-I was isolated from starch degradation products and the residual plant cell wall material by blank filtration and ultrafiltration. The potato RG-I was freeze-dried before further treatment in parallel with enzymatic modification. See below.

Debranching of Rhamnogalacturonan I Side Chains. About 200 mL of 1% RG-I dissolved in 0.2 M phosphate buffer (pH 5.5) was divided into four 50 mL portions in a screw-capped serum bottle. Two of the subsamples were treated with a combination of either α-L-arabinofuranosidase (E.C. 3.2.1.55, *A. niger*) and endoarabinanase (E.C. 3.2.1.99, *A. niger*) or endo-1,4-β-galactanase (E.C. 3.2.1.89, *A. niger*) and β-galactosidase (E.C. 3.2.1.23, *A. niger*) (Megazyme, Wicklow, Ireland) to remove either arabinans or galactans, respectively. The third subsample used all aforementioned enzymes to hydrolyze both arabinans and galactans. The fourth subsample served as a control. An enzyme activity of 0.5 U/mL was used for all enzyme treatments.[14] The samples were incubated at 37 °C for 18 h with constant agitation. The samples were then dialyzed extensively against deionized distilled water for 72 h with frequent water replacement. A dialysis membrane with a molecular weight cut off (MWCO) of 2000 Da was used for separation of the high-molecular-weight pectin. After dialysis, the samples were freeze-dried and subsequently washed with ice cold 96% ethanol to ensure removal of all low-molecular-weight digests. The samples were then redissolved in distilled water, freeze-dried again, and stored for subsequent analysis.

Monomeric Composition. For monomeric composition, freeze-dried sample material was dissolved in demineralized water (1 mg/mL) and hydrolyzed in 2 M triflouroacetic acid (TFA) for 1 h at 120 °C. TFA was subsequently evaporated under vacuum. The hydrolyzed material was dissolved in demineralized water, and each sample was centrifuged before analysis. The monosaccharide composition was analyzed using HPAEC-PAD and employing a PA-20 column (Dionex, CA), as previously described.[14]

NMR Spectroscopy. The solid-state NMR experiments were performed using a Bruker Avance 400 spectrometer (9.4 T) operating at 400.13, 100.62, and 61.42 MHz for ^{1}H, ^{13}C, and ^{2}H respectively, employing a double-tuned solid-state probe equipped with 4 mm (o.d.) spinners. The ^{13}C SP/MAS and CP/MAS spectra were recorded using ^{1}H and ^{13}C rf-field strengths of 80 kHz and a spin rate of 8 kHz. Acquisition times of 48.4 ms were employed, and during (ramped) CP,[15] a contact time of 1 ms was used. High-power ^{1}H decoupling was obtained via the TPPM decoupling scheme.[16] Recycle delays of 4 and 128 s were employed for the CP/MAS and SP/MAS experiments, respectively. These recycle delays were optimized on the dry samples and used for the hydrated samples because previous studies on cell walls demonstrated a reduced longitudinal relaxation time upon hydration.[17] The typical number of scans was 3000 for the CP/MAS spectra and 1200 for the SP/MAS spectra. All spectra were referenced (externally) to the carbonyl resonance in α-glycine at 176.5 ppm. The spectra were apodized by Lorentzian line-broadenings of 10 Hz. Fitting of line widths using a Lorentzian line shape was performed by the built-in procedure in the Topspin 2.1 software. The hydrated samples were prepared by weighing out the powder in the rotor and subsequently adding D_2O using a Hamilton Microliter no. 810 syringe. The needle was inserted into the rotor along the inner wall of the rotor, and the D_2O was slowly released while the needle was removed from the rotor. Prior to acquisition, the hydrated samples had been spinning at 8 kHz for at least 1 h to ensure proper mixing of powder and D_2O. Because of the delicate spinning module in the NMR probe, stable spinning was achieved only when the sample was homogenously distributed within the rotor. This also ensured a proper mixture of powder and D_2O. In this study, D_2O volumes of 20, 25, 34, 44, and 55 μL, respectively, were used. The degree of hydration for each sample is supplied in weight % (= 100% $*m_{D_2O}/(m_{D_2O} + m_{powder})$).

^{2}H SP/MAS (45° flip angle) experiments were recorded using a spin-rate of 2 kHz, 4096 scans, a recycle delay of 1 s, and an acquisition time of 27.3 ms during which ^{1}H TPPM decoupling (80 kHz rf-field strength) was employed. All ^{2}H MAS FIDs were apodized by Lorentzian line-broadenings of 0.3 Hz prior to Fourier transformation. Fitting of the centerband using a Lorentzian line shape was performed by the built-in procedure in the Topspin 2.1 software.

The liquid-state NMR experiments were performed using a Bruker Avance DRX 500 spectrometer (11.7 T) operating at 500.13 and 125.76 MHz for ^{1}H and ^{13}C, respectively. One sample (9.4 mg RG-I in 550 μL of D_2O with 5.8 mM TSP-d4) was analyzed by 2D homonuclear ($^{1}H-^{1}H$) COSY and TOCSY (70 ms mixing time) experiments as well as a heteronuclear $^{1}H-^{13}C$ HSQC experiment using a BBI probe equipped for 5 mm (o.d.) sample tubes.

All experiments were conducted at room temperature.

RESULTS AND DISCUSSION

The monosaccharide compositions of the intact potato derived RG-I (sample NA) and the three enzyme modified RG-Is are displayed in Table 1. The NA has a high content of galactose and consequently a relatively lower content of arabinose. This is in agreement with previous studies of potato pectin composition[14,18] where the side chains of potato RG-I were found to consist primarily of long, linear galactans and branched arabinogalactan I and to a lesser extent highly branched arabinans. The monosaccharide profile agrees well with that reported by Sørensen et al.[19] with due consideration to the different cultivars used. Enzymatic treatment of NA with endoarabinanase and α-L-arabinofuranosidase (sample DA) reduced the arabinose content by 65%, whereas the galactose content was not affected. Treatment with endo-1,4-β-galactanase and β-galactosidase (sample DG) reduced the galactose content by 88% with no effect on the arabinose content. Simultaneous treatment with all four enzymes (sample DB) caused a 69% decrease in the arabinose content and 83% decrease in the galactose content.

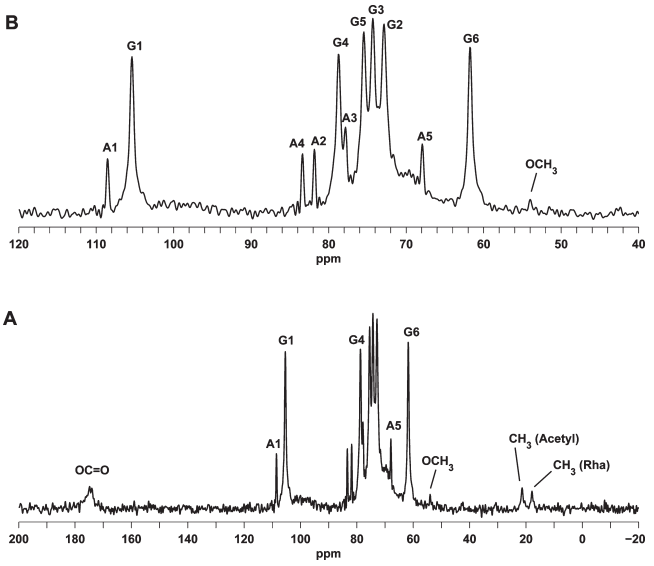


Figure 1. ^{13}C SP/MAS spectrum of native RG-I (NA) at a hydration level of 66.5% displaying the spectral ranges (A) −20 to 200 and (B) 40−120 ppm. The assignment on each spectrum is in accordance with Table 2. A denotes Arabinan, G denotes Galactan, and the number following the letter denotes the position of the corresponding carbon in either the arabinose or galactose units.

Neither galactose nor arabinose could be completely removed by the enzymatic treatments, probably because of steric hindrance of the enzymes near the rhamnogalacturonan backbone, some aspects of which are discussed by Sørensen et al.[19] Galactose did, however, seem to be more easily hydrolyzed than arabinose.

In Figure 1, various spectral regions of the ^{13}C SP/MAS spectrum of NA at a hydration level of 66.5% are displayed. At this level of hydration, a very good spectral resolution is obtained that facilitates detailed assignment of different functional groups present in NA. The assignment is obtained based on a series of 2D homo- and heteronuclear spectra recorded on a water-dissolved DG sample. (See Table 2.) In Figure 1A, the full ^{13}C SP/MAS spectrum is displayed exhibiting resonances from carbonyls (esters or acids) around 175 ppm, methyl groups from either Rha or acetyl groups just below or just above 20 ppm, and from a methoxy group around 54 ppm. Besides these resonances, the main part of the resonances originates from arabinan or galactan side chains and is located between 60 and 100 ppm. Focusing on the region 40−120 ppm (Figure 1B), the assignment of the six carbons in galactan (G1 to G6) and the five carbons in arabinan (A1 to A5) is displayed. In Figure 2, the ^{13}C CP/MAS and SP/MAS spectra of NA in the dry state and at hydration levels of 28.1 to 66.5% are displayed. The Figure shows that the spectral resolution increases as a function of the level of hydration. When comparing the spectra of the dry powder (upper spectra) with the spectra of the more hydrated powders (lower spectra), it is noted that the CP/MAS and SP/MAS spectra display similar features, except for the intensity of the carbonyl carbons around 175 ppm, until a hydration level of 46.4% is obtained. At this hydration level, the anomeric resonances for galactan at 105.3 ppm (G1) and arabinan at 108.2 ppm (A1) are clearly distinguished in the SP/MAS spectrum, whereas the resonance at 108.2 ppm is not present in the CP/MAS spectrum. As hydration is increased, the spectral resolution increases in both types of spectra, but the resonance at 108.2 ppm

Table 2. Assignment of ^{13}C Resonances Based on 2D COSY, TOCSY, and ^{13}C-HSQC NMR Spectra of Diluted RG-I and DG at Room Temperature[a]

residue/carbon	C1	C2	C3	C4	C5	C6	*Cm*[b]
arabinan (α-1,5-Ara)	108.2	81.5	77.4	83.0	67.5		
galactan (*β*-1,4-Gal)	105.3	72.8	74.2	78.6	75.3	61.6	
GalA (backbone)							53.5
Rha (backbone)							17.8
acetyl							21.3
α-galactose (monomer)	92.9	69.0	69.9	70.0			
β-galactose (monomer)	97.1	72.5	73.5	69.3			

[a] Chemical shifts are referenced to an external sample of α-glycine having a carbonyl resonance at 176.5 ppm. [b] *Cm* denotes chemical shifts for methyl groups.

is observed only in the SP/MAS spectra meaning that arabinan is fully mobilized at these levels of hydration in the sense that it is not observable in the CP/MAS spectrum. Because of the higher mobility of the arabinan side chains, the heteronuclear dipolar C−H couplings are averaged to zero, which prevents polarization transfer from ^{1}H to ^{13}C using CP. From the SP/MAS spectrum of the most hydrated sample, it is also noted that the line width of the resonance from A1 (35 Hz) is significantly smaller than the one from G1 (78 Hz). A closer inspection of Figure 1B confirms that the line width for all arabinan resonances are smaller than the resonances from galactan. This observation further supports the fact that arabinan is more mobile than galactan. Similar results have been reported by Foster et al.[11] when comparing ^{13}C CP/MAS and SP/MAS spectra of cell walls extracted from pea and tobacco stems. However, those results are not exactly comparable to ours because their ^{13}C SP/MAS spectra were recorded with a recycle delay of <5 s and therefore not quantitatively accurate.

The line width of the resonance from the carbonyl groups was reduced in the SP/MAS spectra when the hydration level was increased, and in the CP/MAS spectrum at the highest level of hydration, this resonance has almost disappeared. This indicates that the rigidity of the carbonyl group on galacturonic acid (GalA) is reduced, and thereby the CP conditions changed. A plausible explanation for this is increased mobilization due to hydration.

Regarding initial effects of hydration, it is observed that the two partially overlapping resonances at 62.5 and 59.3 ppm in the CP/MAS and SP/MAS spectra of the dry powders (marked by G6) seem to collapse into a single resonance at 61.6 ppm, corresponding to C_6 in hydrated galactan. The line width of this resonance is reduced from ∼250 to 78 Hz when going from dry powder to the highest level of hydration, which indicates increased order of the C_6 carbon by this process. Concerning the initial line narrowing, it is hypothesized that C_6 in the dry powder may adopt two almost equally populated conformations, but addition of water creates a common conformation that is energetically more favorable. Alternatively, the coalescence of the C_6 resonances may be caused by a hydration-induced fast exchange between the two conformations, leading to a single resonance in the spectrum. Furthermore, initial hydration allows for separation of the methyl resonances from rhamnose (Rha) and the acetyl groups located at 17.8 and 21.3 ppm, respectively.

Overall, it is observed that the arabinan side chains are readily hydrated, whereas at least some of the galactan side chains remain

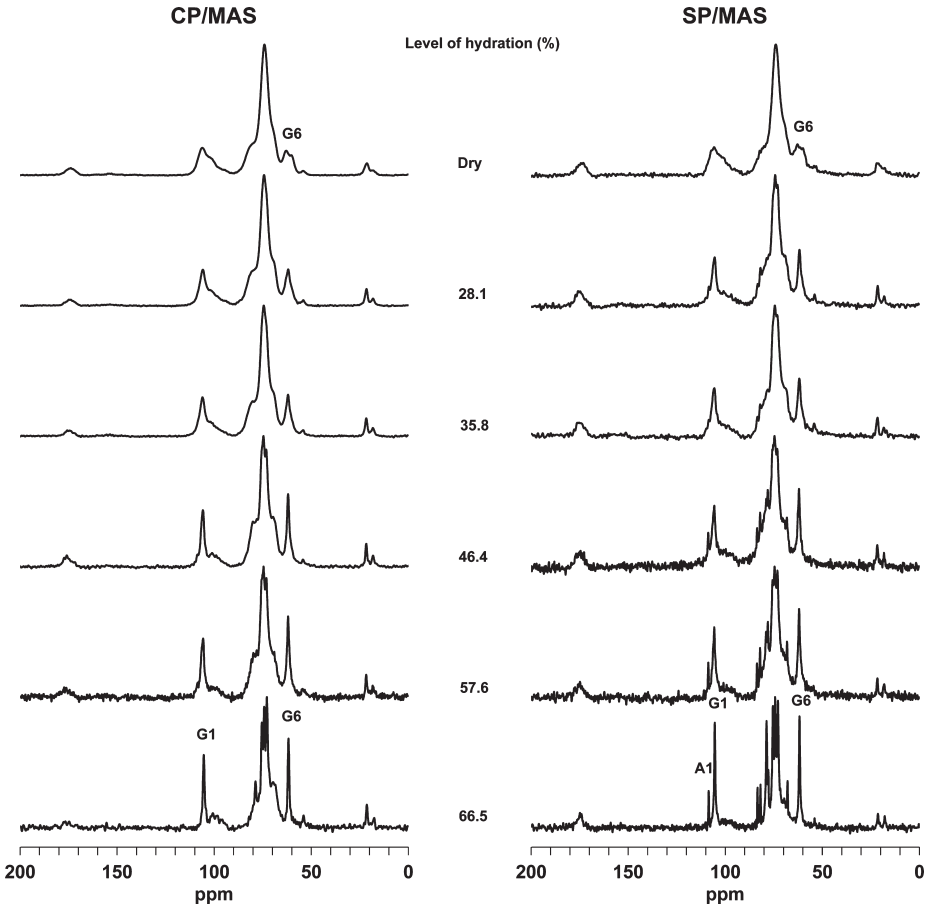


Figure 2. ^{13}C CP/MAS (left column) and SP/MAS (right column) spectra of native RG-I (NA) at different levels of hydration.

sufficiently immobilized for CP, even at the highest level of hydration, whereas the lack of CP efficiency for the arabinan side chains is due to vanishing heteronuclear $^{1}H-^{13}C$ dipolar coupling caused by their high mobility. In addition, the line width of the arabinan resonances is less than half of the one for galactan resonances at the highest level of hydration. Together, these observations indicate that arabinan side chains are present in solution-like environments. This result suggests that the hydration properties of RG-I may be controlled by delicate modification of the amount of arabinan and galactan side chains. From a structural point of view, these properties are related to immobile galactan-rich regions and mobile arabinan-rich regions in RG-I as a consequence of different water accessibility in the β-1,4-linked galactan and α-1,5-linked arabinan side chains. Moreover the linear galactan chains could be more prone to participate in immobilized networks or regions in the sample than the more branched and flexible arabinan chains.[20]

To illustrate the structural impact of side-chain modifications in RG-I, ^{13}C SP/MAS, and CP/MAS spectra of samples DA, DG, and DB were recorded in the dry state and at a hydration level of ~57%. Figure 3 displays these spectra together with the corresponding spectra of the NA sample. Two major trends were observed: (1) the DG and DB samples exhibited similar spectra in the dry state having more intense resonances from carbonyl (~175 ppm) and methyl groups (15−25 ppm) and (2) a broader range of chemical shifts for the anomeric carbons (90−115 ppm) when compared with NA and DA. On the basis of integrals of the acetyl resonance (21.3 ppm) and the carbonyl resonance (175 ppm) in the SP/MAS spectra of the dry powders, it was furthermore observed that the degree of acetylation of GalA was 46 (±1)% in all samples. The spectral resemblance of NA and DA as well as DG and DB reflects that arabinan and galactan side chains constitute ~82% (mol %) in NA and DA but only ~50% in DG and DB. Assuming that the backbone is unaffected by enzymatic treatment, the results in Table 1 indicate that the number of residues per molecule in DA is ~90% of NA, whereas it is only about a third of NA for DG and DB. In the hydrated state, the CP/MAS spectra of NA and DA were almost identical, whereas in the SP/MAS spectra, arabinan side chains (e.g., A1 at 108.2 ppm) are observed only in NA. Likewise, DG and DB were mainly distinguished by the presence of arabinans in DG. Arabinan is also observed in the CP/MAS spectrum of DG. By Table 1, similar NA and DB are characterized by similar arabinan contents (mole %). The Ara/Rha ratio, on the other hand, is ~1.6 in NA and only ~0.5 in DB, whereas it is ~1.6 in DG. Using the fact that the arabinan side chains can be mobilized in both NA and DG and not in DB and taking the Ara/Rha ratio as a measure of the arabinan side chain length, this indicates that a certain length of side chains is required for mobilization at this level of hydration. In contrast, the galactan side chains were clearly mobilized in the hydrated DB. In DB, the arabinose content is about half the rhamnose content, whereas the galactose content is ~1.5 times the rhamnose content. It is hypothesized that shorter side chains permit more direct interactions with the Rha-GalA-backbone and create a less hydrophilic environment.

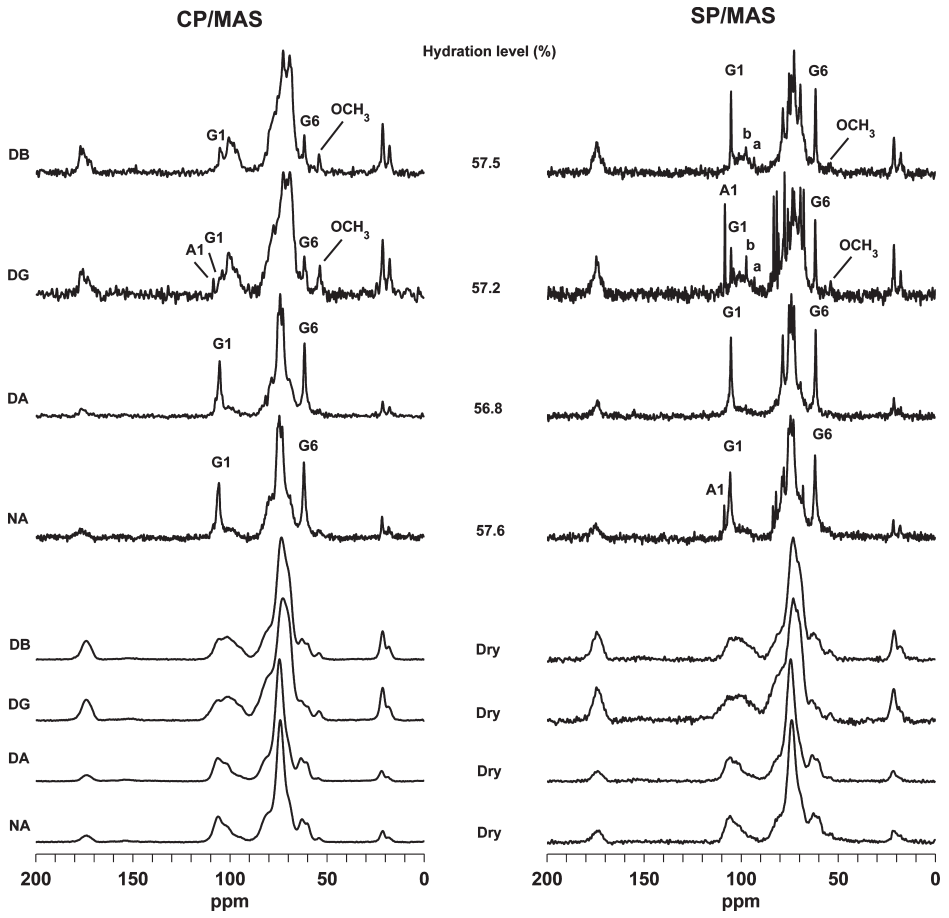


Figure 3. ^{13}C CP/MAS (left column) and SP/MAS (right column) spectra of native RG-I (NA), dearabinated RG-I (DA), degalactanated RG-I (DG), and debranched RG-I (DB) in the dry (lower spectra) state and at a hydration level of ~57% (upper spectra). In these spectra, a and b denote the anomeric carbons in monomer units of α-galactose or β-galactose, respectively.

When comparing the ^{13}C CP/MAS spectra of the hydrated NA and DA with hydrated DG and DB it is observed that a broad resonance around 100 ppm is present in the two latter spectra. In addition, the methoxy resonance (53.5 ppm) in the samples DG and DB is almost as intense as the resonance from C_6 in Gal (61.6 ppm). Because the intensity of the methoxy resonance is very low in the ^{13}C SP/MAS spectra of DG and DB, this demonstrates that the methoxylated GalA's are present almost exclusively in the immobile regions. Moreover, the broad resonance from the carbonyl carbon has a maximum at 176.5 ppm in the CP/MAS spectra of DG and DB, whereas the peak maximum is at 174 ppm in the SP/MAS spectra. According to previous results,[12] these resonances indicate that the carbonyl groups in GalA are mainly present as carboxylic acid. Therefore, it can be concluded that the degree of methoxylation is low and the methoxylated GalA's are primarily located in the immobile regions. This was also observed for NA and DA, but because of the relatively lower content of GalA in these samples, the effects in the spectra were less pronounced. Overall, this indicates a clustering of methoxylated GalA's, and we hypothesize that they are mainly composed of homogalacturonan (HG) extensions of the RG-I backbones.

Besides components in the large polysaccharide, α- and β-galactose monomer units were also observed in the SP/MAS NMR spectra (chemical shifts for anomeric carbons at 92.9 (α) and 97.1 (β) ppm). These were most likely present because of remnants of nondialyzed material or the presence of newly hydrolyzed material caused by low amounts of enzyme becoming activated upon hydration.

Upon addition of D_2O, some of the OH groups in the carbohydrate units may be deuterated such that the deuterium resonances include contributions from OD groups in carbohydrates as well as D_2O and HDO. To study the hydration effect in more detail, we recorded ^2H MAS NMR spectra of NA and DG at selected hydration levels, as shown in Figure 4. No spinning sidebands were observed, even at a spin rate of 2 kHz, revealing that no or only very small anisotropic interactions (chemical shielding anisotropy and quadrupolar interaction) were present. Therefore, the analysis was focused on the hydration-induced changes of the centerband. As anticipated, the line width of the centerband is reduced upon increased hydration. The line width (LW) in NA goes from 330 to 120 Hz when the hydration level is increased from 28.1 to 46.4%, whereas the line width in DG is reduced from 593 to 223 Hz when the hydration level is increased from 35.8 to 46.2%. Differences in line widths reflect differences in mobility and distribution of chemical shifts for deuterons present in D_2O/HDO. The broader line width for DG indicates more immobilized and chemical versatile environments for the deuterons. Presumably, this is due to tighter bound D_2O/HDO than that found in NA. Combined with information from the hydrated DG sample in Figure 2 where both arabinan and galactan is much more abundant in the SP/MAS than in the

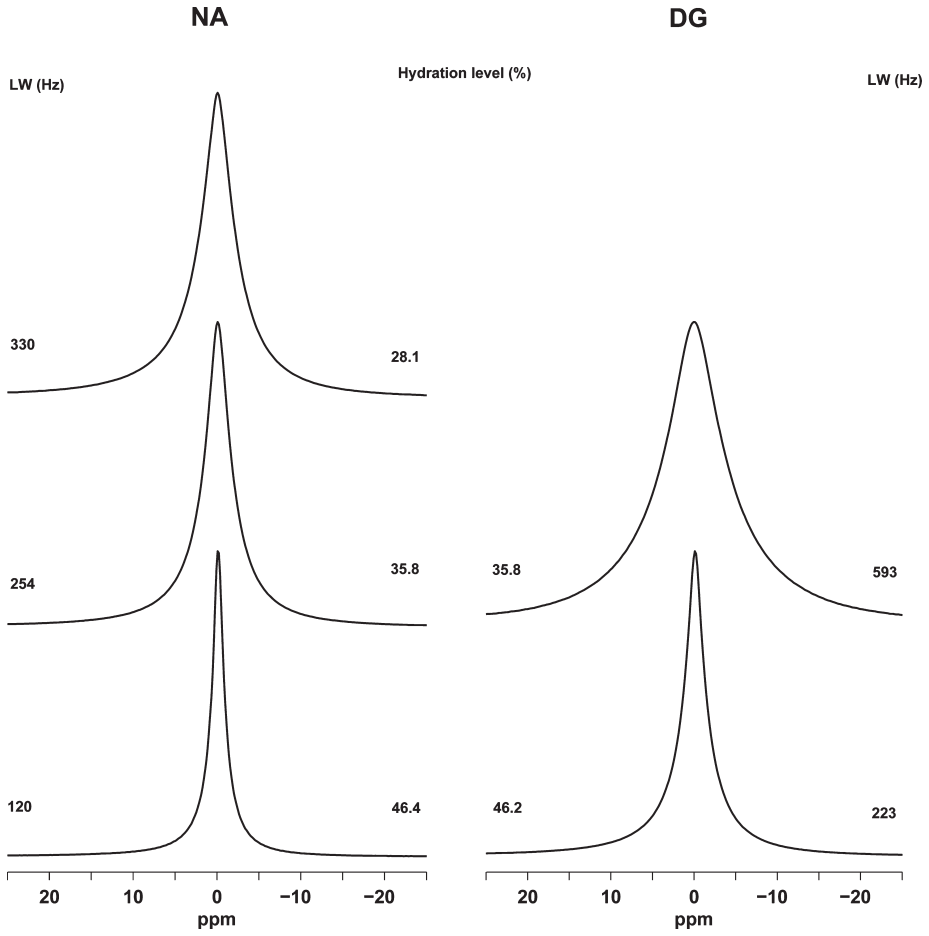


Figure 4. ^{2}H SP/MAS spectra of NA (left column) and DG (right column) at various levels of hydration. LW denotes that line width.

CP/MAS spectrum, this indicates that hydration primarily takes place in the shorter side chains, whereas the backbone is more difficult to hydrate.

Taken together, our results point toward the design of RG-I molecules with specific hydration properties by delicate design of side chains and the arabinan/galactan ratio. Pectins are, in general, employed as thickeners, water binders, stabilizers, emulsifiers, and gelling agents in foods. In contrast with acid-extracted pectin, RG-I is a polysaccharide of poor gelling properties because of its high content of neutral sugars.[21] However, these characteristics make RG-I a potential food ingredient with a different range of applications compared with the present commercially available pectins. Examples from small-scale food applications are already available. Kaack et al.[22] prepared a largely debranched potato RG-I and attributed its functionality as a fat-reducing agent in comminuted meat products to its relatively low water binding capacity. These workers further demonstrated that the same functional properties of the polysaccharide lead to superior performance to other fiber sources in fiber-enriched bread.[23]

CONCLUSIONS

In this study, the hydration properties of RG-I were analyzed by comparative application of ^{13}C CP/MAS experiments that enhance the intensity of carbon resonances from carbons in immobile regions and ^{13}C SP/MAS experiments that provide a correct quantification of all carbon sites. By this approach, it was demonstrated that the arabinan side chains hydrated more readily than the galactan side chains. This points toward preparation of RG-I pectins with specific hydration properties by adjusting the arabinan/galactan ratio in RG-I.

Prior to hydration of arabinan side chains, an initial hydration step related to C_6 in galactan was observed. Hereby a more ordered environment for this carbon was induced.

Exploration of the enzymatically modified RG-I samples, DG and DB, produced similar results, likewise for NA and DA. This indicates that structural and functional characteristics upon hydration are determined by arabinan and galactan side chains for NA and DA, whereas the properties of the Rha-GalA backbone are much more pronounced for DG and DB because of shorter and less abundant side chains.

Analysis of enzymatically modified RG-I's demonstrated that short side chains could be hydrated, but the Rha-GalA backbone was reluctant to hydrate. This is surprising because the backbone carries negative charges but was supported by both ^{2}H and ^{13}C MAS NMR experiments.

The present approach proves to be a strong tool for analysis of hydration properties of intact plant cell walls, polysaccharides, or other biological systems where hydration is of great importance for structure, functionality, or both. In the present study of RG-I, the possibility for tailoring the pectin structure to promote specific hydration properties paves the way for new applications, for example, within food and material science.

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ACKNOWLEDGMENT

Financial support from The Danish National Advanced Technology Foundation through the project *Valorization of Potato Byproducts: New Food Ingredients by an Enzyme-Mediated Extraction Process* is gratefully acknowledged.

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