

This article was downloaded by:

On: 30 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



International Journal of Polymeric Materials

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713647664>

The Application of NMR Techniques to the Study of Polysaccharides from Pulp and Latex of Sorva

Alessandra M. R. Nascimento^a; Maria Inês Bruno Tavares^a

^a Instituto de Macromoléculas Professora Eloisa Mano, Universidade Federal do Rio de Janeiro, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, Brazil

To cite this Article Nascimento, Alessandra M. R. and Tavares, Maria Inês Bruno(2007) 'The Application of NMR Techniques to the Study of Polysaccharides from Pulp and Latex of Sorva', *International Journal of Polymeric Materials*, 56: 2, 115 – 125

To link to this Article: DOI: 10.1080/00914030600735213

URL: <http://dx.doi.org/10.1080/00914030600735213>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

The Application of NMR Techniques to the Study of Polysaccharides from Pulp and Latex of Sorva

Alessandra M. R. Nascimento

Maria Inês Bruno Tavares

Instituto de Macromoléculas Professora Eloisa Mano,
Universidade Federal do Rio de Janeiro, Cidade Universitária,
Ilha do Fundão, Rio de Janeiro, Brazil

*The determination of the chemical components in the pulp and latex of unripe sorva fruit (*Couma utilis*) is very important to control the changes during ripening and storage life of polysaccharides from sorva fruit. According to this, the pulp and latex components have been investigated by nuclear magnetic resonance (NMR), using ^{13}C solution and solid state and proton relaxation times, because this spectroscopy is non-destructive and it is very valuable for the study of complex mixtures. The pulp components were studied by ^{13}C solution NMR through the extraction products, employing solvents with different polarities. The entire fruit was dehydrated in an oven with circulating air at 80°C and analyzed by ^1H and ^{13}C solid-state NMR techniques. The Latex was soluble in chloroform and the ^{13}C solution NMR spectra was recorded, in order to characterize the major chemical components of this mixture.*

Keywords: characterization, NMR, polysaccharides

INTRODUCTION

There is a wide assortment of natural products in the Amazon region, which are very much used by people for different purposes. A native

Received 27 March 2006; in final form 3 April 2006.

The authors thank the Gerência de Química do CENPES/PETROBRAS for the use of solid state NMR spectrometer and the NMR group of Professor Antônio Gilberto Ferreira (IQ/UFSCar) for the ^1H -MAS. The authors also acknowledge CAPES and CNPq for financial support of this work.

Address correspondence to Alessandra M. R. Nascimento, Instituto de Macromoléculas Professora Eloisa Mano, Universidade Federal do Rio de Janeiro, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, RJ, CP 68525, CEP 21945-970, Brazil. E-mail: alessandra@ima.ufrj.br

plant from this region called sorva is one of them; its fruit has a specific aroma/flavor, which is considered exotic. This fruit produces latex when it is unripe, the same latex is found in the stem, this latex is consumed by simple people as milk and also as medicine [1]. Knowing that both fruit and latex materials are complex mixtures, their industry uses depend strongly on a fundamental knowledge of their chemical composition, the changes occurring during ripening, latex processing, time of extraction, Stability, and microbial contamination.

The great majority of studies of these kind of samples involves a hard chemical work of extraction and separation followed by chromatographic analyses, particularly high-performance-liquid-chromatography (HPLC). However, the extent of invasive separation and extraction methods may interface with the biochemistry of the pulp fruit and latex [2–5]. Nuclear magnetic resonance spectroscopy has played an increasing important role in the compositional study of food [5–9], because it is a non-invasive technique with a wide range of applications in natural products as well as food processing, research, and development. Solid state NMR has become an essential technique for food characterization because this technique can not only differentiate between materials in solid form, but it is also an intimate probe of the structural aspects of solid form [6–9].

In this work, the application of NMR to analyze the sorva pulp and its latex was carried out to show the potential of this spectroscopy as a methodology that can be used to chemically characterize such kinds of complex materials. To obtain those responses the authors have employed solution and solid state nuclear magnetic resonance to investigate both whole fruit and latex, because this spectroscopy permits a complete evaluation of these samples using different pulse sequences.

EXPERIMENTAL

Latex and fruit studied in this work were collected in the Amazon State. The latex was collected during the fruit ripening and the unripe fruit were analyzed in their natural state.

The procedure developed to study the major sorva components was:

1. The fruit parts were separated to shell, pulp, and seed;
2. All fruit parts were dehydrate in an oven at 50°C;
3. The dehydrated fruit parts were analyzed by ^1H high resolution MAS;
4. The total dehydrate fruit was analyzed by solid state NMR, employing magic angle spinning (MAS) and cross-polarization magic angle spinning (CPMAS);

5. The total green fruit was treated with different solvents, water, methanol, DMSO, and chloroform in different separation funnels;
6. Four solvent extraction were obtained in item 5 and analyzed;
7. ^{13}C solution NMR analyzes of each solution obtained from item 5 was carried;
8. The green fruit had its latex extracted and the soluble fraction in chloroform was analyzed by ^{13}C -NMR.

To identify the latex components, it was dissolved in chloroform and the ^{13}C solution NMR spectra were registered. To investigate the fruit, the authors decided to extract the fruit components during ripening. Identical quantities of the ripening fruit were put in four different separation funnels and dimethylsulfoxide (DMSO), water (H_2O), chloroform (CHCl_3), and methanol (MeOH) were added in each one to extract the chemical components at ambient temperature, ensuring not to cause any changes in the sample by heating. Figure 1 shows the scheme used for the fruit extraction. The dehydrated fruit was analyzed by routine NMR techniques such as magic angle spinning (MAS), cross-polarization magic angle spinning (CPMAS), and by ^1H high resolution (^1H -HRMAS).

NMR Measurements

The ^{13}C solution analyses were carried out on a VARIAN spectrometer MERCURY 300, operating at 75.4 MHz for ^{13}C and ambient

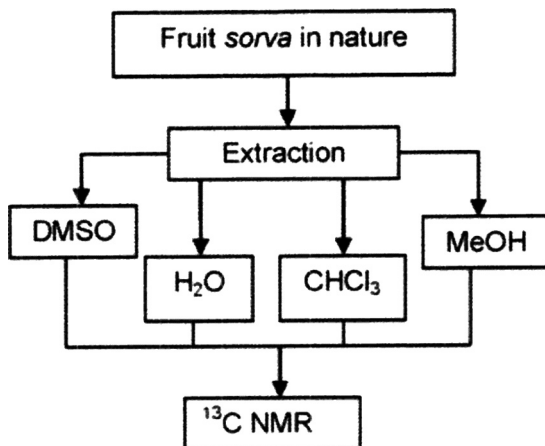


FIGURE 1 Diagram of the approach used for extraction the components of sorva.

temperature probe, applying a single pulse sequence, with 1.59 s for acquisition time, 18888.8 Hz of spectral width, 1 s of recycle delay between 90° pulses and the NOE effect was removed. In the DEPT (Distortionless Enhancement by Polarization Transfer) pulse sequence the J (coupling constant) value was 140 Hz. For the APT (Attached Proton Test) pulse sequence the same conditions of single pulse sequence were used with J as 140 Hz.

All solid state NMR spectra were obtained on a VARIAN spectrometer INOVA 300 MHz, operating at 75.4 MHz for ^{13}C , and ambient temperature probe. The sample was placed in a rotor with diameter of 7 mm and spun at speeds of 5 kHz in the magic angle spinning (MAS).

The acquisition conditions for MAS technique were 0.3 s of recycle delay, 30.000 Hz of spectral width and 0.04 s of acquisition time. For the cross-polarization magic angle spinning (CPMAS) the acquisition conditions were 2 s of recycle delay, 30.000 Hz of spectral width, 0.04 s of acquisition time and 1 ms of contact time with a B_1 strength as 40 KHz.

The ^1H -HRMAS spectrum was obtained on a BRUKER DRX 400 spectrometer operating at the following conditions: spectral width: 5668,9 Hz; acquisition time: 1,8 s; pulse width: 45°; recycle delay: 1 s and number of transients: 128. The type of processing was zero filling and line broadening: 0.

RESULTS AND DISCUSSION

The methodology used to characterize the main chemical components of the latex and pulp fruit employed both solution and solid state NMR analyses, because this spectroscopy allows an evaluation of the sample behavior in different time scales.

Solution NMR Results

The ^{13}C solution NMR analysis of the fruit latex (Figure 2) was carried out after it was extracted during ripening. From the ^{13}C solution NMR spectrum, signals could be identified as coming from cis-1,4,-polyisoprene, which were located at ~ 27 ppm (CH_3) ~ 30 ppm (CH_2) ~ 33 ppm (CH_2), ~ 125 ppm ($\text{CH}=\text{}$), and ~ 135 ppm ($\text{C}=\text{CH}$) and other components like polysaccharides, whose resonance signals are located at 60–65 ppm ($\text{CH}_2\text{-OH}$), 68–82 ppm (CH-OH) and 90–106 ppm (C-O-C); flavanoids with signals located at 120–124 and 142–152 ppm and triacylglycerides with long aliphatic chains and the carbonyl signals located at 172 and 174 ppm.

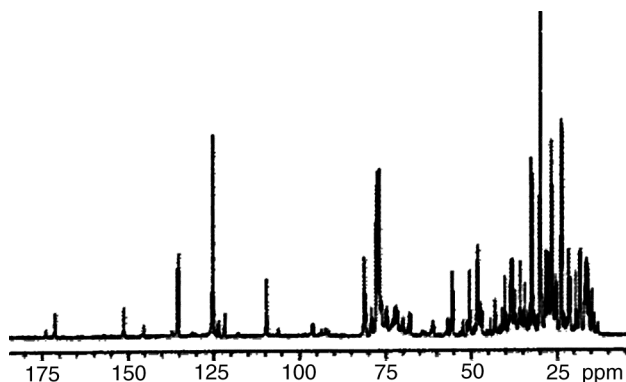


FIGURE 2 ^{13}C NMR solution of sorva latex in deuterated chloroform.

Whole fruits were put in four separation funnels and solvents such as chloroform, water, dimethylsulfoxide, and methanol were separately used in each funnel at room temperature to extract the chemical components, trying to characterize the major components of the fruit. The extraction products were analyzed by normal ^{13}C solution NMR and some interesting data were obtained.

Figure 3 shows the ^{13}C solution NMR spectrum of the dimethylsulfoxide extract. Table 1 shows three ranges of major components detected, which probably come from saccharides and polysaccharides. Figure 4 shows the DEPT ^{13}C for the dimethylsulfoxide extract.

From chloroform extract, the resonance signals detected were attributed to triacylglycerides, cis-isoprene, and other minor

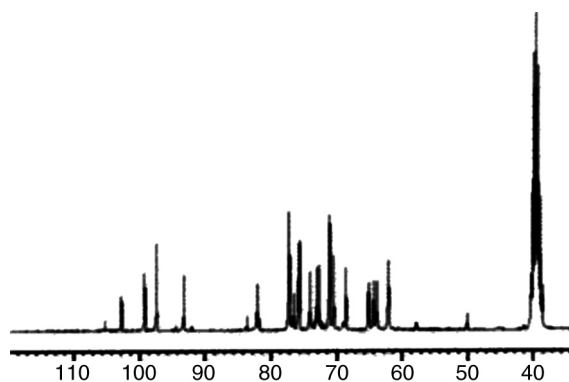


FIGURE 3 ^{13}C NMR solution of DMSO extract from sorva fruit.

TABLE 1 ^{13}C NMR Assignment of the Extract Obtained by DMSO Extraction

δ (ppm)	Carbon type
50	O-CH ₃ *
60-66	CH ₂ -OH
68-86	CH-OH
90-100	C-O-C
100-106	C anomeric*

*Confirmed by DEPT pulse sequence, see in text below for definition.

components, which were also collected (Figure 5). Table 2 shows the assignments of the components extracted from chloroform. From this solvent different components were extracted and at least four ranges

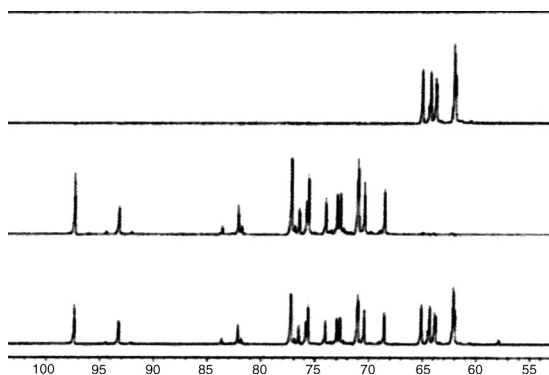
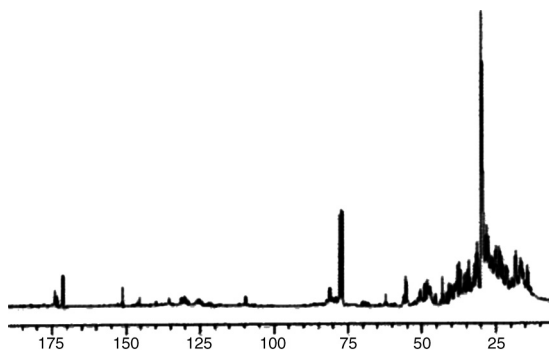
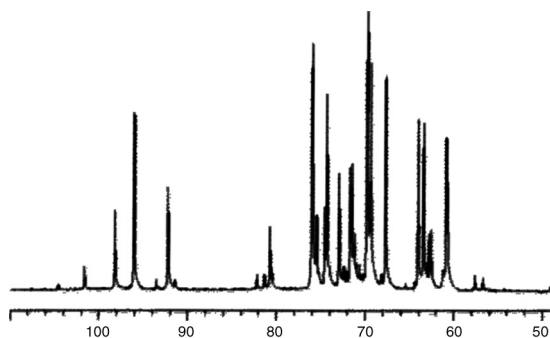
**FIGURE 4** DEPT ^{13}C NMR solution of DMSO extract from sorva fruit.**FIGURE 5** ^{13}C NMR solution of CHCl_3 extract from sorva fruit.

TABLE 2 ^{13}C NMR Assignment of the Extract Obtained by CDCl_3 Extraction

δ (ppm)	Carbon type
10–50	Aliphatic saturated carbons, CH_3 , CH_2
27	CH_3 , cis-isoprene
50–90	$\text{CH}_2\text{-OH}$ and CH-OH from polysaccharides
100–140	Unsaturated carbons and/or aromatic carbons
125	Unsaturated CH of cis-isoprene
135	Unsaturated C_α of cis-isoprene
140–160	Unsaturated carbons from the triacylglycerides
170–185	Carbonyl groups

could be assigned. An intense signal located at 29 ppm was attributed to CH_2 main chain. The carbonyl groups located at 170 to 173 ppm are likely derived from aliphatic ester of triacylglycerides such as oleic, linoleic, and palmitic whereas the carbonyl of carboxylic acid was assigned at 182 ppm.

The ^{13}C NMR spectrum of the extract obtained from water (Figure 6) shows the same pattern of DMSO (see Figure 3) and methanol spectra (Figure 7). Therefore, some additional signals located from 10 to 50 ppm were attributed to saturated carbons belonging to aliphatic triacylglycerides. This behavior indicates that these three solvents have the same extraction power. In conclusion, the use of water, methanol, and DMSO as solvents allows the extraction of the same type of fruit components. In this sense, these data indicate that in unripe sorva the two major components polysaccharides and triacylglycerides exist, as well as cis-isoprene.

**FIGURE 6** ^{13}C NMR solution of water extract from sorva fruit.

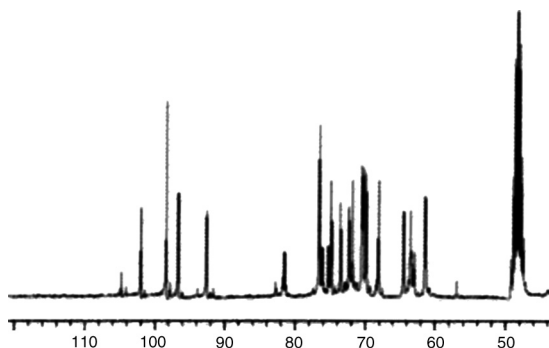


FIGURE 7 ^{13}C NMR solution of MeOH extract from sorva fruit.

Solid State Results

The ^{13}C MAS and CPMAS techniques were employed to obtain information on the chemical components that belong to domains with different molecular mobilities. Thus, the MAS pulse sequence was recorded with short recycle (0.3 s) delay between 90° pulses, in order to observe only the compounds that constitute the mobile domain, such as cis-isoprene. Table 3 shows the assignments of the components found by MAS pulse sequence.

The ^{13}C CPMAS technique was used to detect all type of sample content, principally in the rigid domains. Table 4 exhibits the assignments of all resolved carbons determined from ^{13}C CPMAS

As can be seen from the tables, no much difference was detected from the chemical shifts of both ^{13}C NMR. Therefore, comparing both ^{13}C MAS (Figure 8) and ^{13}C CPMAS (Figure 9) spectra, the chemical shift assignments do not show differences; the major signals detected belong to polysaccharides, which constitute one large rigid domain that probably controls the relaxation process. The mechanism of T_1 relaxation was controlled by dipolar-dipolar process. However, signals from triacylglycerides, which constitute the mobile domain, were also

TABLE 3 ^{13}C MAS Assignment of Entire Sorva Fruit

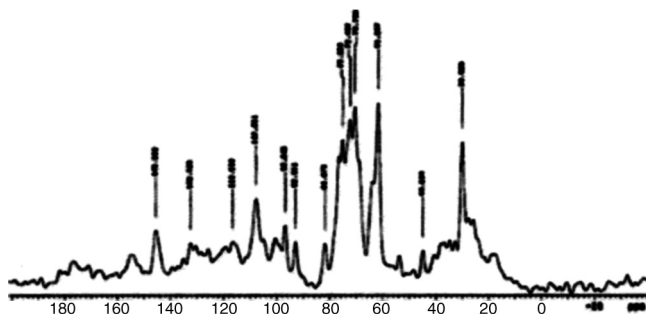
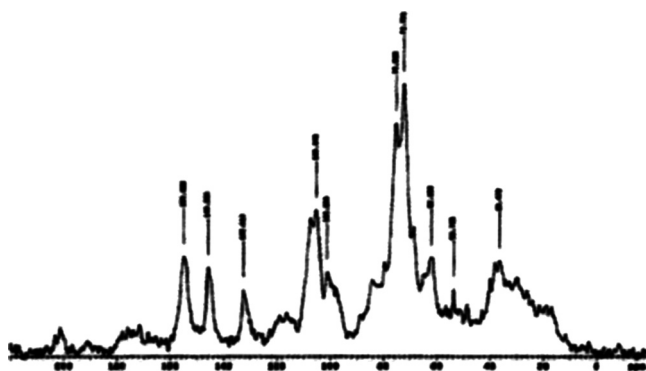
δ (ppm)	Carbon type
10–40	Saturated carbons
60–90	$\text{CH}_2\text{-O}$, CH-O
90–110	C-O-C
145	Unsaturated carbon

TABLE 4 ^{13}C CPMAS Assignment of Entire Sorva

δ (ppm)	Carbon type
10–40	Saturated carbons
60–90	$\underline{\text{C}}\text{H}_2\text{-O}$, $\underline{\text{C}}\text{H-O}$
90–110	C-O-C
130–155	Unsaturated region

detected. Thus the ^{13}C MAS and CPMAS NMR spectra are not completely similar, which confirms the heterogeneity of the unripe fruit.

The entire fruits were dried in an oven with circulating air at 80°C for 24 h and after that a ^1H -HRMAS technique was applied to

**FIGURE 8** ^{13}C NMR MAS of entire sorva fruit.**FIGURE 9** ^{13}C NMR CPMAS of the entire sorva fruit.

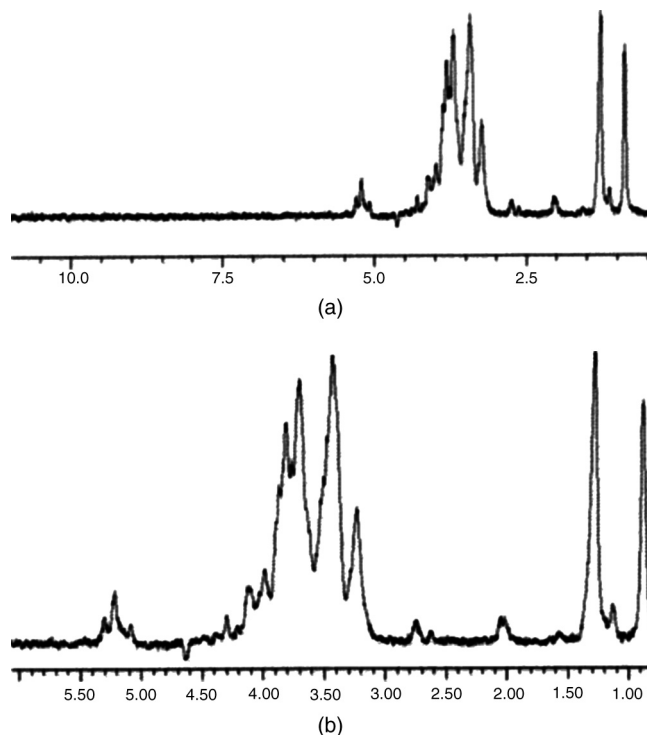


FIGURE 10 (a) ^1H HRMAS of the entire sorva fruit. (b) Expansion of the ^1H HRMAS of the entire sorva fruit.

investigate the main components and also to confirm the data obtained by carbon -13 solid state analyses, because solid state ^1H -HRMAS NMR provides a powerful technique to identify the different protons belonging to distinct domains. The ^1H -HRMAS NMR spectra are shown in Figures 10a and b. Figure 10a shows the entire spectrum and Figure 10b shows the expanded spectrum. The signals detected from 0.9 to 2.0 ppm belong to the long aliphatic chains of the triacylglycerides, whereas the signals observed from 3.0 to 5.4 ppm were attributed to polysaccharides (signal located at ≈ 5.4 ppm was assigned to the hydrogen linked to anomeric carbon the ring carbon attached to a ring oxygen and a bridged oxygen and the signals from 3.6 to 4.6 ppm were attributed to the hydrogens linked to $\text{CH}_2\text{-OH}$ and CH-OH groups). From these results it can be confirmed that the unripe fruit were mainly comprised of polysaccharides and triacylglycerides, in addition to the latex.

CONCLUSIONS

According to the main purpose of this work, the combined use of ^{13}C NMR solution, high resolution ^1H -HRMAS, and solid state techniques provided a useful methodology, which can be used as a strong tool in the chemical investigation of both fruit and latex of sorva. The developed methodology can be used as initial characterization and it permits the monitoring of structural changes in the course of ripening.

REFERENCES

- [1] Falcão, M. A., Clement, C. R., and Gomes, J. B. M., *Acta Bot. Bras.* **17**, 4 (2003).
- [2] Sacchi, R., in *Annals of The Fifth International Conference on Applications of Magnetic Resonance in Food Science*, Aveiro, Portugal, (2000), Vol. 1, 120.
- [3] Gidley, M. J. and Bociek, S. M., *J. Am. Chem. Soc.* **110**, 3820, (1988).
- [4] Gil, A. M. (2000). An Insight into the structure of foods using ^{13}C and ^1H magic angle spinning (MAS) NMR: Application to wheat dough, *The Fifth International Conference on Applications of Magnetic Resonance in Food Science*, Aveiro, Portugal, Vol. 1, 43–53.
- [5] Harris, R. K. (2000). "Recent advances in solid state NMR," *The Fifth International Conference on Applications of Magnetic Resonance in Food Science*, Aveiro, Portugal, Vol. 1, 1–11.
- [6] Tavares, M. I. B., Bathista, A. L. B. S., Silva, E. O., Filho, N. P., and Nogueira, J. S., *Carbohydrate Polymer* **53**, 213 (2003).
- [7] Conte, P., Spaccini, P., Piccolo, A., *Prog. Nuc. Mag. Res. Spec.* **44**, 215 (2004).
- [8] Tavares, M. I. B., Bathista, A. L. B. S., Costa, P. M., Silva, E. O., Filho, N. P., and Nogueira, J. S., *J. Appl. Polym. Sci.* **92**, 2151, (2004).
- [9] Costa, P. M., Tavares, M. I. B., Silva, E. O., Bathista, A. L. B. S., Nogueira, J. S., Filho, N. P., Ferreira, A. G., Barison, A., Daolio, C., and Vizzotto, L., Submitted for publication.