Solid State and Proton Relaxation NMR Study of *Dipteryx alata* Vogel

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ABSTRACT: The knowledge of polysaccharides obtained from fruit seed and its chemical characteristics are important to evaluate their final properties, as well as their uses and benefits. Solid state nuclear magnetic resonance (NMR) has been used to evaluate the behavior of the samples, since NMR permits to evaluate both chemical and dynamic molecular behavior of polysaccharides, because they are amorphous and heterogeneous. The NMR analyzes were carried out using high and low field NMR techniques. The results obtained from high field showed the main chemical constituents present in the seeds and seed flour. Through low field NMR different domains with their own molecular mobility and interactions were observed; due to the measurements of proton spin-lattice relaxation time (T_1H). According to the results obtained for Cumbaru seed, solid state NMR showed to be a powerful source to characterize fruit seed flour. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 116: 50–54, 2010

Key words: NMR; polysaccharides; relaxation time; *Dipteryx alata* Vogel; cumbaru seed

INTRODUCTION

Dipteryx alata Vogel belongs to Fabaceae family and it is the scientific name of a fruit, whose trees is found in Central Brazil, principally in Minas Gerais, Goiás, Distrito Federal, Mato Grosso, and Mato Grosso do Sul. This species is known popularly for baru in Minas Gerais; barujo, coconut-beans, and cumbaru in Mato Grosso; cumarurana, emburena brave, and cumaru wood in the other states. As far as characteristic is concerned this fruit is described to have a pulp-rich in proteins, and seeds—mainly constituted by oil, starch, fiber, and protein. Cumbaru seed is also eatable, nutritional and has medicinal properties.[1–5] It is known that the applications, properties and benefits of these seeds have relationship with its composition and chemical characteristics. That is the reason why it is important to have as much as knowledge is possible of fruit seed. Then, some techniques such as x-ray, electronic scanning electron microscopy (SEM), thermal analysis and nuclear magnetic resonance (NMR) have been used to evaluate these types of materials. NMR can also give information on chemical and dynamic molecular behavior.[6,7]

The NMR spectroscopy in the solid state has many techniques that are available to study chemical characterization at the molecular level of materials. ^{13}C cross polarization and magic angle spinning (CPMAS) and ^{1}H relaxation times have been very much used to study food and systems like polysaccharides, proteins, sugars and so on, because of their relevance in studying bulk samples. The ^{13}C CPMAS NMR is helpful to understand structural differences between samples that have similar nature. The proton spin-lattice relaxation time in the rotating frame ($T_{1\rho}H$), which can be determined by the resolved carbon-13 decay, during the variable contact time experiment (VCT), can inform on sample homogeneity, chemical components and chains interaction and amorphous polymers. The ^{1}H high resolution (^{1}H HR-MAS) is a technique that allows us to obtain ^{1}H-NMR spectra in the solid state, using magic angle spinning with the same resolution as obtained for liquids. The use of this technique permits better hydrogen assignment and can be used as a part of a methodology to better evaluate the carbohydrates behavior.[7–16]

The low field NMR is another technique that can be used together with the others techniques mentioned to determine proton spin-lattice relaxation time (T_1H) parameter, using the traditional inversion-recovery pulse sequence. The values of this parameter allow us to provide detailed

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information on mobility at the molecular level. The wide use of NMR techniques comes from the time scale and, of course, the responses that you obtain.[17,18]

According to this context, the aim of this work is to obtain chemical structural and dynamical information from cumbaru seed before and after oil extraction to contribute to better understand its behavior and consequently to improve their applications and benefits. To achieve this purpose, we have used solid state NMR spectroscopy, through the determination of proton spin-lattice relaxation time in the rotating frame parameter, measured by the resolved carbon-13 decay, during the VCT experiment with increasing the contact time. And also by measuring the values of proton spin-lattice relaxation time in the laboratory, applying the traditional inversion-recovery pulse sequence (180° - τ - 90°), using low field NMR spectrometer. In the present work these NMR techniques were chosen, because they will provide chemical characterization of materials; the understanding of structural differences, due to the domain formation, and also detailed information on molecular mobility at the molecular scale.

EXPERIMENTAL PROCEDURE

Sample preparation

The cumbaru seeds had been extracted from the pit mechanically with an aid of lathe, and then they had been peeled manually. After that procedure the seeds were worn out in a mill of balls. In a second step the oil seed was extracted using hexane in soxhlet equipment, for 24 h. The last step was to dehydrate, in which the seeds where heated at 70°C for 48 h in an oven, to obtain the cumbaru seed flour. To avoid water absorption after this procedure the samples had been kept in a dissector.

Characterization by high field NMR

^{13}C CPMAS analyzes

The powdered samples were analyzed in NMR spectrometer of 400 MHz (Varian – Unity Plus 400), using the basic techniques: cross polarization and magic angle spinning (CPMAS) beyond the variation contact time experiment (VCT). They have been placed in zirconium rotor and the ^{13}C-NMR experiments in the solid state were carried out according the following conditions: frequency of ^{13}C nucleus: 100.4 MHz; acquisition time: 0.02 s; spectral window: 100 kHz; 90° pulse width: 4.7 μs; recycle delay: 2 s; range of contact time: 200–6000 μs; number of transients: 3200. The MAS technique was also carried out using short recycle delay (0.3 s) to observe only the mobile region, which in this case is the seed oil.

^{1}H HR-MAS analyzes

The powdered samples had been placed in rotor of zirconium and added some drops of D_2O. The ^{1}H HR-MAS spectra were obtained on a BRUKER DRX 400 spectrometer operating at these 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.

Characterization by low field NMR

The powdered samples (4–6 g) had been placed in appropriate pipe and the measurements were carried out on a MARAN ultra 23 (Resonance, Oxford-UK), operating at 23 MHz (for protons) and equipped with an 18 mm variable temperature probe. The proton spin-lattice relaxation (T_1H) values were determined directly by the traditional inversion-recovery pulse sequence (180° - τ - 90°) at 27°C, the instrument software calibrated the 90° pulse of 7.5 μs automatically. The amplitude of the FID was sampled for forty τ data points, ranging from 10 to 10e^6 μs, with 4 scans for each point and 5 s of recycle delay. The distributed exponential fittings as a plot of relaxation amplitude versus relaxation time were performed by using the software WINDXP®, which come with the equipment. The relaxation values and relative intensities were

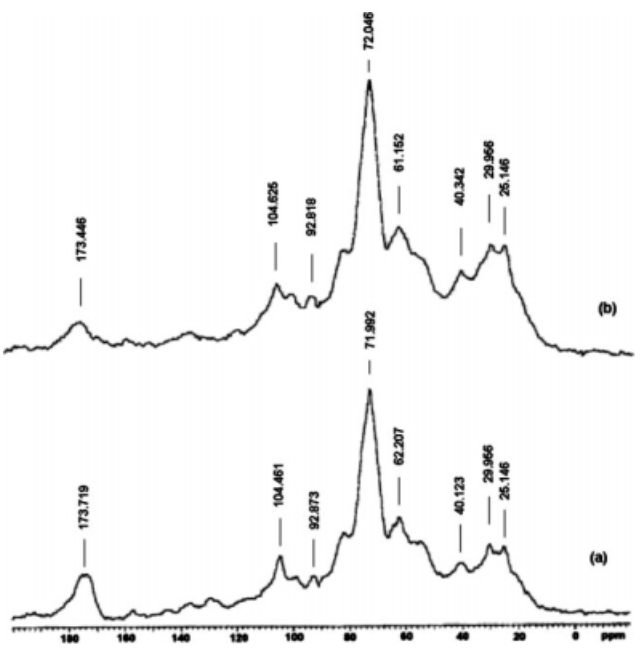

Figure 1 ^{13}C-NMR spectra obtained by CPMAS for: (a) cumbaru powdered seed and (b) cumbaru seed flour.

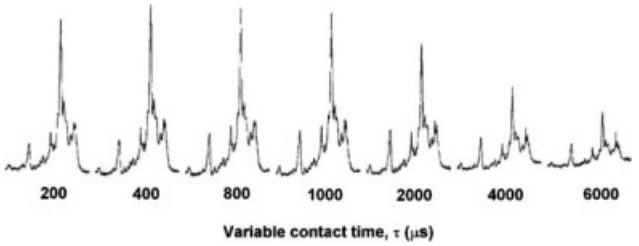

Figure 2 NMR ^{13}C CPMAS series of spectra for cumbaru powdered seed.

obtained by fitting the exponential data with the aid of the programs WINFIT®, which is a commercial program and DPP® developed in our laboratory (which is process of registration).

RESULTS AND DISCUSSION

Characterization by high field NMR

^{13}C CPMAS analyzes

The ^{13}C MAS spectra, in special conditions, were recorded and no signal refer to the oil was detected after the oil extraction process being done in the seed, showing that the procedure was efficient.

The ^{13}C CPMAS NMR analysis is a technique that observes all carbon-13 nuclei, in special the carbons placed in the rigid domains. The spectra obtained for the seeds and seed flour (Fig. 1) show broad signals, which indicate that the samples are amorphous and/or have low crystallinity degree. In these spectra a signal located at 173 ppm refers to carbonyl group; the signals located at the range of 60–105 ppm refer to carbons linked to oxygen, as the anomeric carbon and the region from 25 to 40 ppm refer to CH_3, CH_2, and CH groups. These signals presented similar chemical shift according to the studies of other polysaccharides.[19–21]

The VCT experiment (Figs. 2 and 3) allows us to obtain a profile of spectra distribution with the increase of contact timescontact time; optimum contact time, first indication of heterogeneity and the values of $T_{1\rho}H$ for each resolved carbon (Table I). From Figures 2 and 3 the major intensity signals are

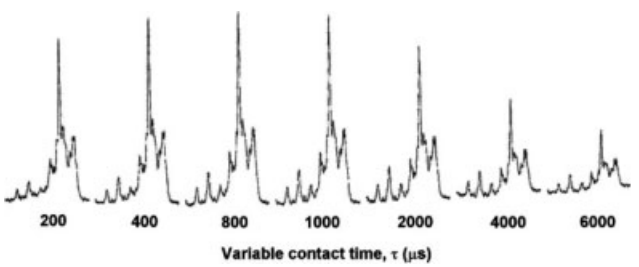

Figure 3 NMR ^{13}C CPMAS series of spectra for cumbaru seed flour.

TABLE I
$T_{1\rho}H$ Values Obtained From High Field NMR for Cumbaru Worn Out Seeds and Cumbaru Seed Flour in its Respective Chemical Shifts

Sample	δ (ppm) 173	104	92	72	62	40	29	25
	$T_{1\rho}H$ ($\times 10^3$ μs)							
Worn out seeds	5	4	2	4	4	4	5	4
Seed flour	11	5	2	3	3	5	4	4

concentrated between 800 and 2000 μs. Thus, the optimum contact time was the 800 μs, for both seeds. From the analyzes of ^{13}C nucleus in the VCT experiment, the region with low molecular mobility presents signals with more intensity in short contact times, which is an indication that the samples present molecular rigidity.

From Table I it was observed a significant difference on the $T_{1\rho}H$ values for the carbonyl region, comparing cumbaru seed and seed flour. It occurs because of the oil present in a major quantity in the seed before the extraction, promoting multiple interactions. For the seed flour, had to the low quantity of oil, constituents' interactions diminished and new interactions among the chains are created, which implies modifications in the molecular mobility. The $T_{1\rho}H$ values obtained for the others chemical shifts do not present significant differences, since then, intermediate $T_{1\rho}H$ is what predominates, because of the materials heterogeneity and rigidity.

^{1}H HR-MAS analysis

The spectra obtained by seeds and seed flour (Figs. 4 and 5) show signals in the region from 5.0 to 5.5 ppm, which refer to hydrogen linked to anomeric carbon; from 4.0 to 4.5 ppm referring to hydrogen present in CH—OH groups; and from 3.0 to

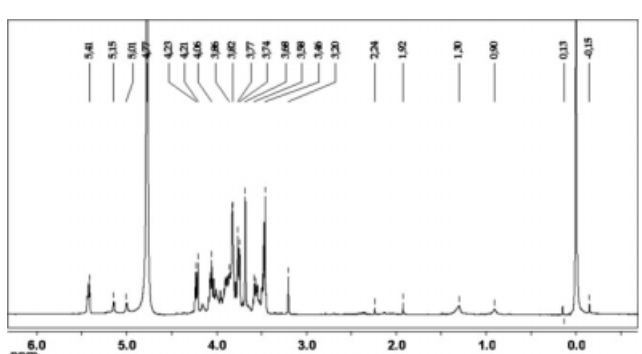

Figure 4 ^{1}H-NMR spectrum of cumbaru powdered seed obtained by HR-MAS.

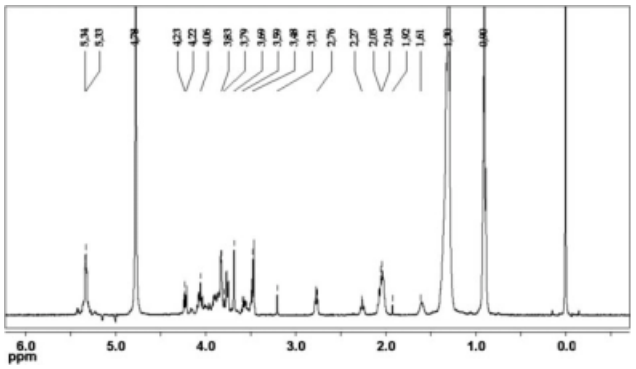

Figure 5 ^{1}H-NMR spectrum of cumbaru seed flour obtained by HR-MAS.

4.0 ppm, which refer to hydrogen in CH_2—OH groups. The spectrum obtained for the seeds show signals with much higher intensity in the region from 0.5 to 3.0 ppm that refer to hydrogen in the oily fraction. These signals are according to the studies already done for other polysaccharides.[9–12]

Characterization by low field NMR

The results obtained by the measurement of proton relaxation times showed that the entire seed and powdered seeds presented three regions with different molecular mobility (Fig. 6), two with high mobility as assigned in Table II, which are mentioned to the water proton nucleus (0.05 and 0.3 $\times$ 10^3 μs) and oil proton nucleus (1.5 and 4 $\times$ 10^3 μs); and the third region that represents the region with more restricted mobility, which belongs to the protons nucleus from water/polysaccharides and proteins (125 and 144 $\times$ 10^3 μs).

In the case of seed flour, two regions was observed, one with high mobility, which is assigned to the water proton nucleus (1.5 $\times$ 10^3 μs), due to their molecular mobility in this type of samples and another, with more restricted mobility, which is related to the water protons nucleus interacting with the polysaccharides and proteins (84 $\times$ 10^3 μs) intracellular that presents less molecular motions, because of strong multiples intermolecular and intramolecular interactions.

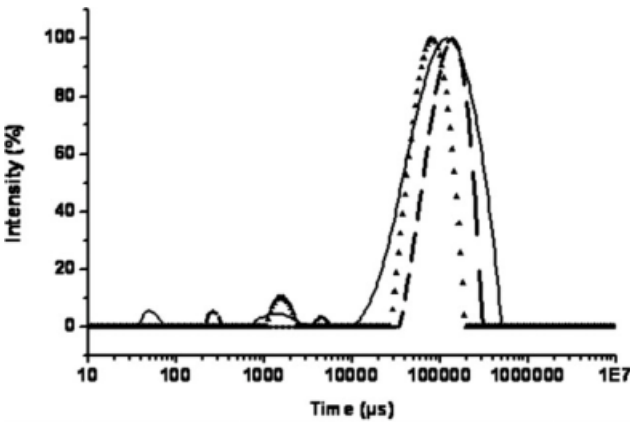

Figure 6 Distribution curves obtained by low field NMR for (–) entire seed, (--) powdered seed and (▲) seed flour.

TABLE II
T_1 Values Obtained by Low Field NMR for the Samples

Sample	T_1H ($\times 10^3$ μs)	Intensity (%)	$T_1H_{(1\ FIT)}$ ($\times 10^3$ μs)
Entire seed	0.05	1	97
	1.5	2	
	125	97	
Worn out seeds	0.3	3	115
	4	4	
	144	93	
Seed flour	1.5	7	73
	84	93	

Comparing the T_1H values obtained for the rigid domains of the samples, it can be observed that the seed flour presented low value indicating an increase in the sample molecular mobility after the oil extraction, because of the decrease in the intermolecular interactions, creating more freedom to the chains, which confirms $T_{1\rho}H$ results, showing that the oil causes multiple interactions with the constituents. In relation to the intensities, it was observed that the rigid domains are presented with higher intensity, comparing to the others. Because they are responsible for controlling of the relaxation process, due to the fact their relaxation processing is longer since they have more interaction among the molecular chains. Analyzing the results obtained by $T_1H_{(1FIT)}$, it was observed a decrease in the time relaxation obtained for seed flour comparing with the other samples, because of the diminish in the intermolecular chains interactions, these statement was also confirmed the results showed by $T_{1\rho}H$.

CONCLUSIONS

The NMR in the solid state showed to be a good source to characterize both chemical components and the molecular mobility of seed fruit, as we have done, this work, for cumbaru seed.

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