

# <sup>13</sup>C-NMR Study of *Dipteryx Alata Vogel* Starch

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**ABSTRACT:** Natural starches obtained by fruit seeds have been a subject of studies because of their applications, as a consequence of their chemical properties. As it is known, the fruits depend on the geographic region. Because of this, several factors affect the seed starches nature, such as granules size, morphology, components, and composition. Thus, to better understand starch properties, it is necessary to investigate the chemical behavior of these factors. This work focused on the chemical components and molecular dynamic behavior of the *Dipteryx Alata Vogel* (cumbaru)

starch, because this fruit is very popular and is used in abundance for people because they think that it is good for some kinds of diseases. The main chemical components and the molecular dynamics of starch from the *Dipteryx Alata Vogel* were studied by applying solid-state <sup>13</sup>C nuclear magnetic resonance spectroscopy (NMR). © 2004 Wiley Periodicals, Inc. *J Appl Polym Sci* 92: 2151–2154, 2004

**Key words:** NMR; starch; characterization

## INTRODUCTION

Starches are always stored in the form of granules and they present a complex structure. Despite several studies,<sup>1–4</sup> the molecular organization of starch granules is not very well established yet, because both granules size and morphology vary very much with starch botanical source, region, and season time. Therefore, some physical properties of the starch granule are well known; they are semicrystalline, constituted by polysaccharides (amylose and amylopectin), and insoluble in cold water. The study of chemical components and molecular dynamic of the native starch is very interesting because new information can be obtained from the use of different points of investigation according to the methodology used to make it. Solid-state NMR spectroscopy permits the evaluation of the sample in different time scales.<sup>5</sup> NMR spectra are sensitive to the nature of the material, including heterogeneity. Much chemical and physical microstructure information can be obtained because of the application of distinct pulse sequences used to generate subspectra of components in different time domains and to allow the observance of structural changes of starch in the solid state, and it is nondestructive.<sup>1–5</sup> The focus of this work is to investigate the

main chemical components and the molecular dynamic behavior of starch obtained from the seed of *Dipteryx Alata Vogel*, to use it, for example, in pharmacological drugs. The cumbaru starch was studied in bulk and was characterized by solid-state nuclear magnetic resonance techniques; the analyses were carried out by using different solid-state NMR techniques, such as magic-angle spinning (MAS),<sup>5–8</sup> cross-polarization magic-angle spinning (CPMAS),<sup>9–13</sup> and the proton spin-lattice relaxation time in the rotating frame ( $T_1^H\rho$ )<sup>5–13</sup> determined by two experiments, variable contact time (VCT) and delayed contact time (DCT) experiments. In summary, the objective of this work was to evaluate the main polysaccharides presented in this starch and the molecular dynamic behavior in relation to the organization of dry starch granules obtained from *Dipteryx Alata Vogel* seed.

## EXPERIMENTAL

### Starch extraction

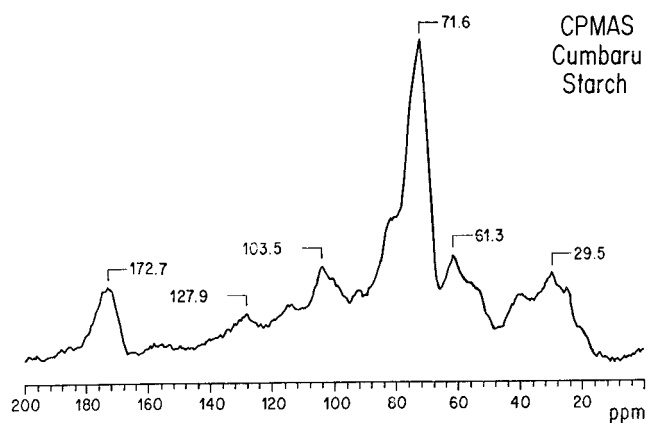
The starch of the cumbaru fruit was obtained after seed-oil extraction. The seed was put into a Soxhlet extractor (Mato Grosso, Brazil) and the oil was extracted with acetone. The cumbaru starch was powdered, dried in a vacuum oven for 24 h, and kept in a dissector. Infrared spectroscopy was used to identify the presence of water, oil, and other impurities.

### NMR measurements

All solid spectra were obtained on a Varian Inova 300 spectrometer, operating at 75.4 MHz for <sup>13</sup>C. All solid

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**Figure 1** CPMAS  $^{13}\text{C}$ -NMR spectrum of cumbaru seed starch.

experiments were carried out at ambient probe temperature with high-power decoupling. A zirconium oxide rotor of 7 mm diameter was used to acquire the NMR spectra at spinning rates of 5.5 kHz. The  $^{13}\text{C}$ -NMR spectra were obtained by using MAS, with a short recycle delay (0.3 s) between  $90^\circ$  pulses and 5000 scans; in the CPMAS experiment, with a strength of spin-locking field  $B_1$  of 40 kHz, the recycle delay was 2 s and 3200 scans were used. For the variable contact-time experiment, the lock time ranged from 200 to 8000  $\mu\text{s}$ . Proton  $T_{1\rho}$  values were determined from the intensity decay of  $^{13}\text{C}$  peaks with increasing contact times and also from the delayed contact-time experiment, with a spin-lock time varying from 200 to 8000  $\mu\text{s}$ . The CPMAS with dipolar dephase, with a dephasing time,  $\tau$ , of 40  $\mu\text{s}$ , was also recorded.

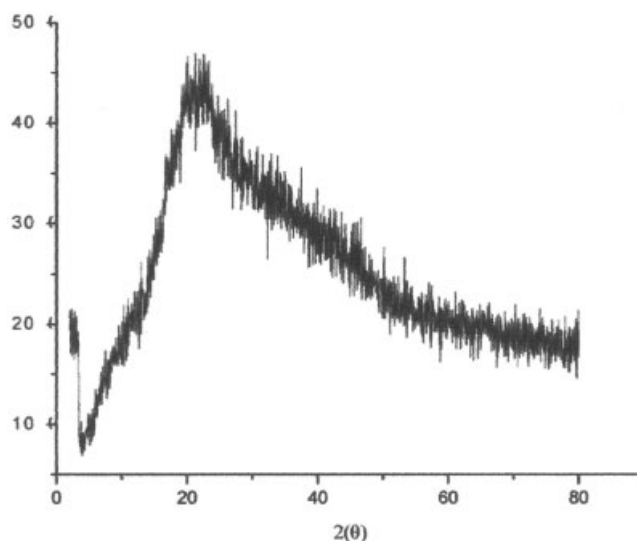
*X-rays* The X-ray analysis was carried out on a Rigaku miniflex diffractometer, operating at the  $\text{CuK}\alpha$  wavelength (1.542  $\text{\AA}$ ).

## RESULTS AND DISCUSSION

The molecular mobility and organization studies of starch extracted from the seed of *Dipteryx Alata Vogel* were characterized by  $^{13}\text{C}$  solid state, using basic techniques such as MAS and CPMAS, and proton spin-lattice relaxation time in the rotating frame.

**TABLE I**  
Chemical Shift of Main Compounds Found in Cumbaru Starch

Carbon	$\delta$ (ppm)
$\text{C}=\text{O}$	172.7
$\text{C}-\text{O}-\text{C}$	103.5
$\text{C}_{(4)}-\text{OH}$	88.2
$\text{C}_{(2,3,5)}\text{H}-\text{OH}$	71.6
$\text{C}_{(6)}\text{H}_2-\text{OH}$	61.3
$\text{CH}_2$	29.5

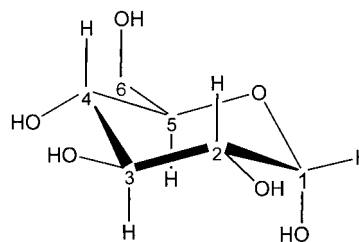


**Figure 2** X-ray diffraction pattern of cumbaru seed starch.

The cumbaru seed starch, before any treatment, was evaluated by the basic MAS  $^{13}\text{C}$  recorded with short recycle delay (specified in Experimental) between  $90^\circ$  pulses, before oil extraction. The spectrum showed sharp signals located at 172 ppm ( $\text{C}=\text{O}$ ), 130–127 ppm ( $\text{C}=\text{C}$ ), 68 ppm ( $\text{CH}-\text{O}$ ), 62 ppm ( $\text{CH}_2-\text{OH}$ ), and from 34 to 13 ppm (aliphatic carbons  $\text{CH}_2$ ,  $\text{CH}$ , and  $\text{CH}_3$ ). These signals were attributed to the seed oil. The seed oil was extracted (see Experimental) and characterized by solution  $^{13}\text{C}$ -NMR; its structure was derived from a triacylglycerol with a long linear chain.<sup>14–16</sup>

After oil extraction, the dry starch cumbaru granule had its  $^{13}\text{C}$  MAS spectrum recorded and, according to the specific acquisition conditions used, only the mobile carbons should be detected. As a result of this experiment, no signal was detected, as was expected. Starches do not have a mobile region in this time scale, which is an indication that this starch is a rigid sample.

The  $^{13}\text{C}$  CPMAS NMR spectrum (Fig. 1) of the dry starch granules showed a combination of features of spectral crystalline and amorphous materials, because



**Figure 3** representation of starch unity.

**TABLE II**  
 $T_1^H\rho$  Values, as a Function of Chemical Shift,  
 Obtained by VCT

Type of carbon	$\delta$ (ppm)	$T_1^H\rho$ (ms)
C anomeric	103.5	3.1
CH—OH	71.6	2.9
CH <sub>2</sub> —OH	61.3	4.0

of signal width and chemical shift. Six main NMR signals were detected. Table I shows the frequency of all resolved resonances of cumbaru seed starch, obtained by using the  $^{13}\text{C}$  CPMAS at the optimum contact time (800  $\mu\text{s}$ ). The chemical shifts are derived from  $\alpha$ -1,4-D-glucose and also from protein.

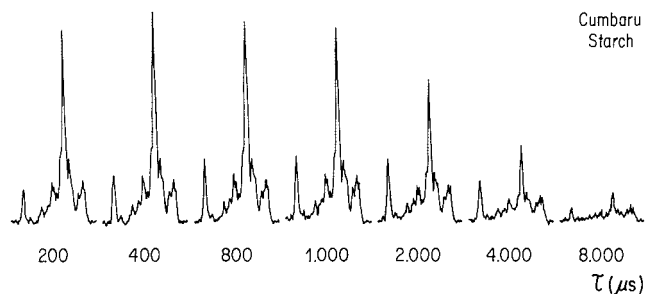
The X-ray diffraction patterns confirm that the cumbaru starch showed a behavior of semicrystalline sample, and the crystalline region was derived predominantly from A-form and amorphous phase (Fig. 2). The starch unity is represented in Figure 3.

The  $^{13}\text{C}$  CPMAS NMR spectrum of starch, obtained from fruit seed, presents some similarities in the polysaccharide regions with starches derived from cereals, such as maize and potato, for example, especially in the 60- to 64-, 68- to 77-, and 94- to 105-ppm ranges.<sup>1-4</sup> However, for the 80- to 86-ppm range, a better resolution can be observed for the fruit seed starch. The resonance line of C<sub>4</sub> can be detected as separate from the C<sub>2,3,5</sub> sites. These starch differences can be attributed to the amylose/amylopectin ratio and also from the crystalline and nonordered conformations. From the signals, line width, and chemical shift values, the cumbaru starch seems to be a heterogeneous polysaccharide, which probably presents, at least, two domains with distinct molecular mobility. Two types of polysaccharide structures can be estimated such as single chain (amorphous) and double helix (crystalline). Similar to the study of molecular organization in starch,<sup>1</sup> in the  $^{13}\text{C}$  CPMAS spectrum of cumbaru starch, the C-1 resonance also covers a range of 100- to 104-ppm chemical shift, which suggests that at least part of the amorphous component has a well-defined conformation.

Focusing on the dynamic behavior and molecular organization of cumbaru starch, VCT and DCT experiments were carried and  $T_{\text{CH}}$  and  $T_1^H\rho$  parameters for all resolved carbons were determined. Tables II and III

**TABLE III**  
 $T_1^H\rho$  Values, as a Function of Chemical Shift,  
 Obtained by DCT

Type of carbon	$\delta$ (ppm)	$T_1^H\rho$ (ms)
C—O—C	103.5	3.4
CH—OH	71.6	2.9
CH <sub>2</sub> —OH	61.3	3.0



**Figure 4** VCT  $^{13}\text{C}$  decays of the cumbaru seed starch.

show the values for  $T_1^H\rho$  parameter, using VCT and DCT experiments, respectively.

From both measurements, the  $T_1^H\rho$  values did not change significantly and a single value was found for each resolved carbon. This behavior shows that this starch presents one rigid domain, probably the crystalline one, that controls the relaxation process.

Comparing the values range of  $T_1^H\rho$  found for cumbaru starch to the cereals starches<sup>1</sup> (3–4 ms), they present the same range; therefore,  $T_{\text{CH}}$  values are not similar. The  $T_{\text{CH}}$  values determined for C—O is 230  $\mu\text{s}$ , the  $T_{\text{CH}}$  values determined for CH—OH is 140  $\mu\text{s}$ , and the  $T_{\text{CH}}$  values determined for CH<sub>2</sub>—OH is 90  $\mu\text{s}$ .

The VCT decays (Fig. 4) are single exponentials, because the intermolecular interaction is strong and nonspin diffusion is observed; as a consequence, the VCT decays represent a typical decay of rigid amorphous polysaccharide, which is corroborated by  $T_1^H\rho$  values.<sup>17</sup>

$^{13}\text{C}$  CPMAS with dipolar dephase spectrum showed three signals, one from carbonyl group located at 172.7 ppm and two aliphatic signals located at 29.4 ppm (CH<sub>2</sub> of main chain) and 23.2 ppm (CH<sub>2</sub>). These signals probably derived from a type of gluten (Fig. 5); we cannot now specify the type of protein, because it is not our purpose at the moment. Therefore, it demonstrates that this starch presents a much larger quantity of protein than those obtained from cereal sources.

## CONCLUSION

According to the NMR data acquired, it was possible to obtain useful information on chemical compounds and molecular dynamics from this type of starch seed, using the solid-state routine NMR techniques and the relaxation parameter. From the results, it was evaluated that this starch is a nonordered compound.

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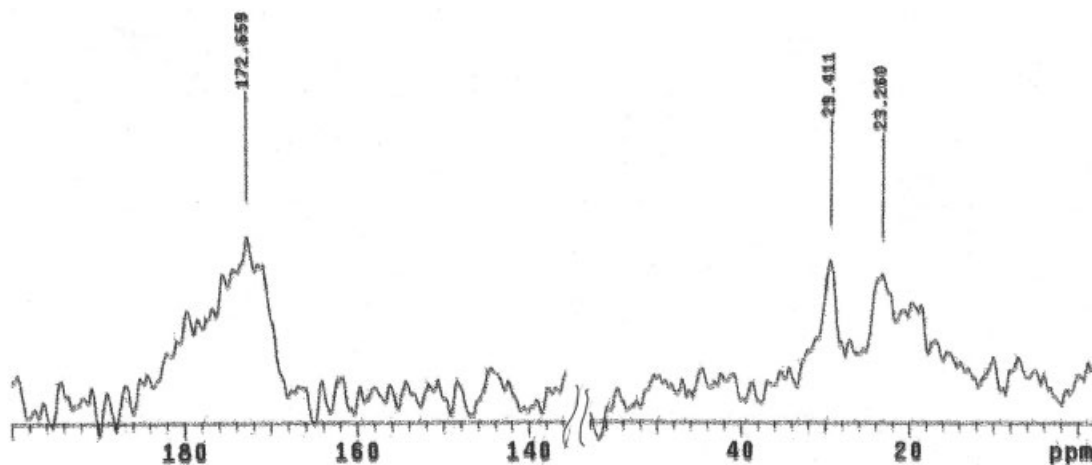


Figure 5 CPMAS  $^{13}\text{C}$ -NMR spectrum with dipolar dephase of cumbaru seed starch.

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