High Resolution NMR Study of Tropical Fruit Seed Starches

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ABSTRACT: The nuclear magnetic resonance spectroscopy (NMR) studies of starches obtained by fruit seeds such as melon and watermelon were carried out as an analytical methodology to understand the molecular dynamic behavior. This study was also accompanied by the conventional X-ray diffraction and thermal analysis. The NMR results give us a complete behavior of the samples at the molecular level. Thus, the information obtained from NMR will complement the data obtained from X-ray and thermal analysis allowing us having responses on samples structure and dynamical behavior. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 973–977, 2007

Key words: NMR; biomaterials; molecular dynamic

INTRODUCTION

Polysaccharides and their derivatives are very much used in food processing technology. Among polysaccharides, starch is often used as an inherent natural ingredient and it has been studied because it has many applications, such as thickening agent, gelling agent, and food packing, because, of course, it is a biodegradable polymer, it comes from renewable source and has low cost.[1–4] Starches are generally found in granule form, the size of the granules vary from 1 to 100 μm (maize, 2–30 μm; wheat, 1–45 μm; potato, 5–100 μm). The crystallinity degree varies up to 30%, and the crystallization process can generate different crystalline forms such as A, B, and C.[3–5] Starch granule stability depends on the arrangement of atoms in the polysaccharides structure, according to distribution of the intra- and intermolecular forces. Structural characteristics such as chain conformation and intermolecular associations influence the physicochemical properties of starches' chains. Not many studies have been applied to fruit seed starches comparing to starches derived from wheat, corn, potato, tapioca and rice, for example. The seeds' starches contain protein in different levels, vitamins, especially vitamin E, minerals, and photochemical protective. In these starches we can also find different carbohydrate polymers that cannot be digested by human being. These carbohydrate fibers contribute to the well-hydrated bulk and there is a strong indication that these fibers may have a protective effect against colon cancer, as well as the heart diseases. Soluble fiber can reduce cholesterol levels because it binds with bile, which is made up of cholesterol. It also delays the time taken for digested food to move through the intestines, e.g., slows down the digestion of carbohydrates such as sugars, which results in better glucose tolerance.[3–5] Thus, the improvement in the knowledge of fruit seed starch in relation to composition and its chemical characteristics are very important to evaluate the final properties of them, such as processability, as well as their applications. Some conventional techniques such as X-ray, electronic scanning microscopy, thermal analysis, and nuclear magnetic resonance have been used to evaluate starches and polysaccharides chemical and dynamic molecular behavior.

Solid state nuclear magnetic resonance spectroscopy has many techniques that are available to study chemical characterization of different materials. ^{13}C cross polarization and magic angle spinning (CPMAS) and ^{1}H relaxation times have been very much used to study foodstuff and systems such as polysaccharides, proteins, sugars, and so on, because of their relevance in studying bulk samples.[6–10] The ^{13}C CPMAS NMR is very helpful to understand structural differences between samples that have similar nature.[8–10] 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

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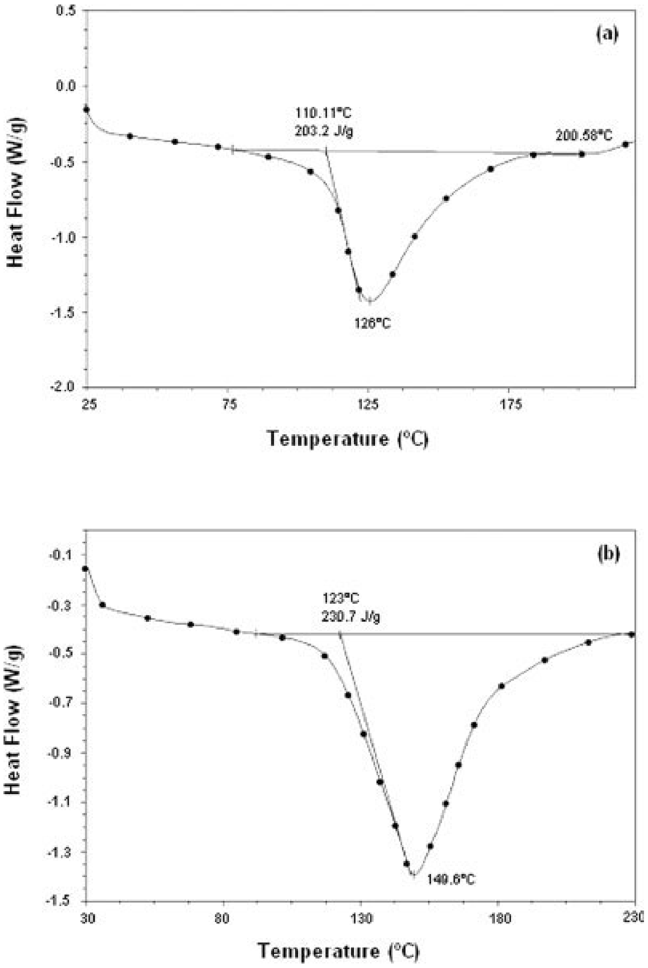

Figure 1 DSC curve: (a) melon starch seed and (b) watermelon starch seed.

the variable contact time experiment (VCT), can inform on sample homogeneity, chains interaction and for amorphous polymer such as starch the heterogeneity according to the domains formation, which can be constituted by ordered and nonordered polysaccharides chains.[7,8] The ^{1}H high resolution (^{1}H HR-MAS) is a technique that allow us to obtain ^{1}H NMR spectra at solid state, using magic angle spinning. The use of this technique permits a better hydrogen assignment and can be used as a part of a new methodology[9] to better understand the starches behavior. The wide use of NMR techniques comes from the time scale and, of course, the responses that you get.[5–10]

The main purpose of this work is to characterize the starches obtained by fruit seed of melon and watermelon, to know their main chemical component, such as oil, proteins, polysaccharides as well as the molecular dynamic of these starches, employing solid state NMR techniques, together with X-ray measurements to help us understand starches' behavior.

EXPERIMENTAL

The melon and watermelon seed starches were kindly supplied from a research group located at Universidade Federal do Mato Grosso state in Brazil, which has an agreement with a Brazilian industry who prepare flour from starch seed. The characterization of these native starches was accompanied by X-ray, thermal analysis, ^{13}C CPMAS, VCT, $T_{1\rho}H$, and ^{1}H HR-MAS. The moisture content of these starches was also determined.

The samples weighted were put on an oven at 70 °C for 24 h to remove the absorbed water. After that, they were kept in a dessicator to reach the ambient temperature; this procedure was done three times. The analyses were procedure in the dried samples.

The melting temperature was determined for each sample in a DSC V4.4E (TA instruments) and the conditions were as follows: Nitrogen atmosphere:

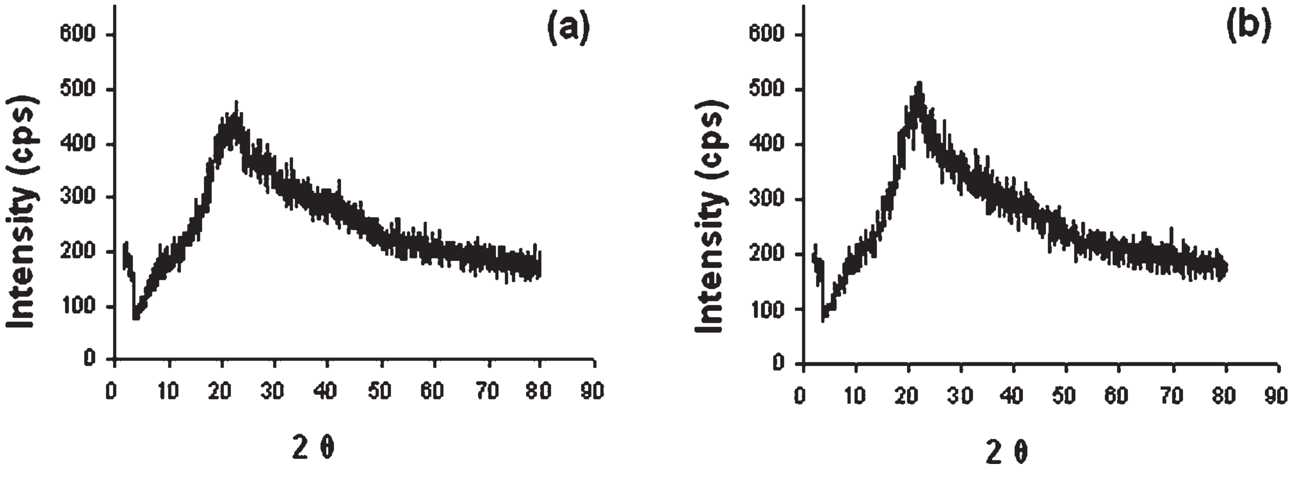

Figure 2 X-ray diffraction pattern: (a) melon starch seed and (b) watermelon starch seed.

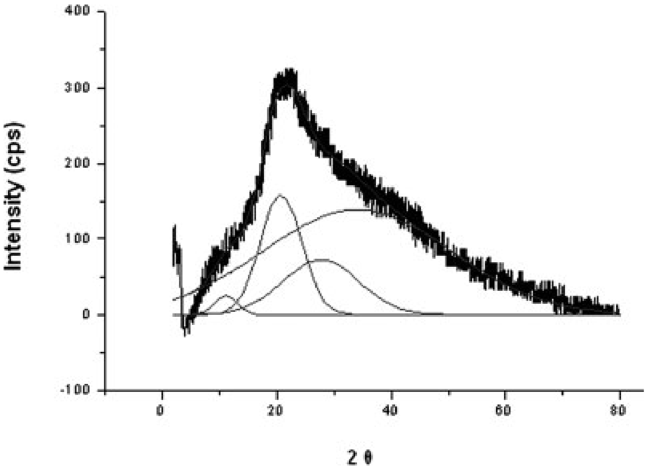

Figure 3 Crystallinity determination of melon starch seed.

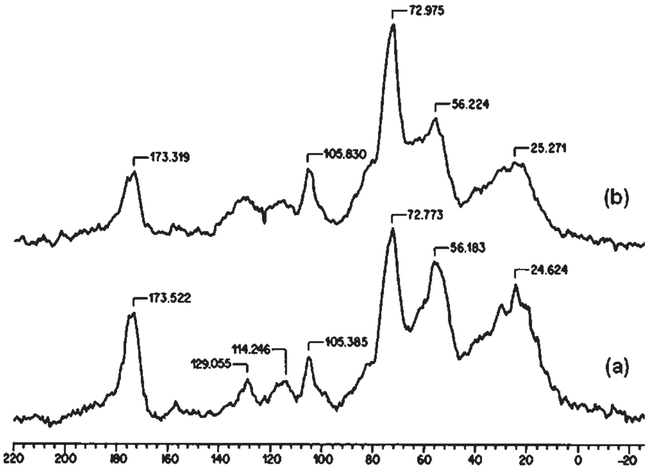

Figure 4 ^{13}C CPMAS spectra: (a) melon starch seed and (b) watermelon starch seed.

30 mL/min; heating velocity: 10 °C/min; temperature range: 10–210 °C.

The X-ray diffraction patterns were obtained with a Rigaku Miniflex diffractometer, operating at the Cu kα wavelength (1542 Å). The crystallinity degree was obtained by Origin program.

The ^{13}C CPMAS spectra were obtained on a VARIAN INOVA 300 spectrometer, applying such conditions as: spectral width: 100 kHz; acquisition time: 1.8 s; pulse width: 90°, recycle delay: 2 s, the spinning rate was 6 kHz; and number of transients: 20,000. The processing used was zero filling and line broadening: 50.

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.

RESULTS AND DISCUSSION

Melon and watermelon starches presented similar moisture content, which was determined as 8.4%. The moisture contents of the samples are in the range of the values determined for starches, such as cereals for example. Before all analysis, the samples were submitted to the same conditions to be dried and all analyses were done in the dehydrate samples, as it is very well known that absorbed water interferes very much in the relaxation parameter.

The melting temperature (T_m) for melon and watermelon seed starches can be determined from the Figure 1(a,b), respectively. The melting temperature found for melon starch was 126 °C and for watermelon starch was 149.6 °C. The difference in these values comes from the fact that the water dispersion in the matrix interferes in the crystallization mode. This is a consequence the crystal sizes growth differently, in the melon starch the crystal sizes are smaller than the watermelon starch, the quantity of internal water in this starch promotes a local nucleation induced by solubilization that is responsible for the growth of crystal, generating big and more perfect crystals comparing to the melon starch.

Melon and watermelon starches' X-ray diffraction patterns can be seen in Figure 2. Both starches presented similar diffraction patterns, which can be associated to an A-type of crystalline form. However, the amorphous phase is very broad in both starches types, because of water dispersion mode in these samples.

The crystallinity degree was determined by Origin program and it was given by the peaks intensity of the diffraction patterns using a deconvolution method (Fig. 3). The crystallinity degree for melon starch was 18%. The same proceedings were done for watermelon starch, but it was not possible to

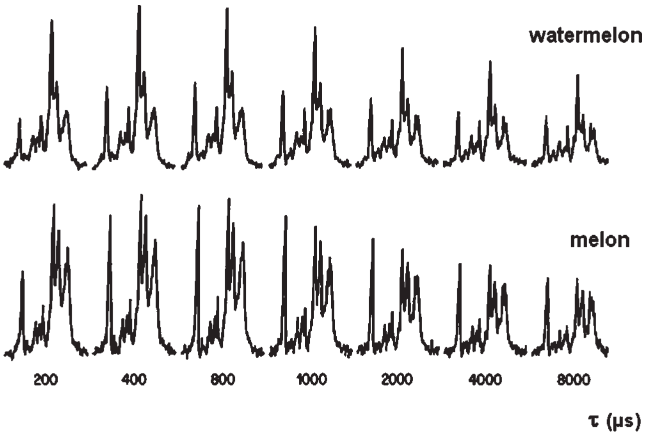

Figure 5 VCT experiment for melon starch seed and watermelon starch seed.

TABLE I
Proton Spin-Lattice Relaxation Parameter from Melon Starch Seed and Watermelon Starch Seed, Determined from Each Resolved Carbon-13 Decay During the VCT Experiment

	$T_{1\rho}H$ (ms)		
Sample	105.8	72.7	56.2 (δ ppm)
Melon	2.8	4.4	4.1
Watermelon	8.4	5.4	2.2

determine its degree of crystallinity, probably because in this starch sample the water dispersion is different and can cause changes in both crystalline and amorphous starch structure. This behavior may confirm that the crystal forms, packing, and arrangements of the crystals in the watermelon starch are different comparing to the melon seed starch.

From ^{13}C CPMAS NMR spectra of melon starch and watermelon starch (Fig. 4), different signals can be readily assigned. The spectral region from 40 to 60 ppm is refereed to **C**H_2—OH (C-6), from 70 to 80 ppm belongs to **C**H—O (C-2,3,5), and from 100 to 110 ppm is dominated by anomeric **C**H—O (C-1), for the carbon atoms of the anhydroglucose unit. Therefore, two broad signals centered at 25 and 173 ppm derived from both glycoproteins and glycolipids were also observed. Comparing both spectra, ^{13}C CPMAS spectrum melon starch showed signals with better resolution in comparison to the watermelon starch spectrum. This fact comes from the difference in the morphology, which is relative to the crystallinity packing and amorphous phase dispersion that was not evidenced from the X-ray diffraction patterns. From the ^{13}C CPMAS spectrum melon starch presents crystalline phase morphology different from watermelon starch, which is reflected also from the value of melting temperature (T_m), in spite of both starches presented a wide amorphous phase.

The variable contact-time during the cross-polarization experiment can generate a dependence of polarization transfer with contact-time according to eq. (1). In this experiment the relaxation time in the rotating frame, $T_{1\rho}H$, can be determined from decay of all resolved carbons and this parameter permits us to get useful information from the intermolecular interaction, phase dispersion, domain formation, and sample homogeneity. As the measurement of $T_{1\rho}H$, from VCT, is influenced by the polarization transfer, it also is dependent of sample rigidity and inform on molecular behavior. Generally, mobile samples take long to be polarized, while rigid sample takes short time to be polarized. Figure 5 compares the VCT decay for melon and watermelon seed starches. The $T_{1\rho}H$ data are listed in Table I.

$$I = 1/[1 - \exp(-\tau/\mathrm{TCH})]\exp(-\tau/T_{1\rho}H) \quad (1)$$

From the values of relaxation parameter obtained from the resolved carbon signals of polysaccharides

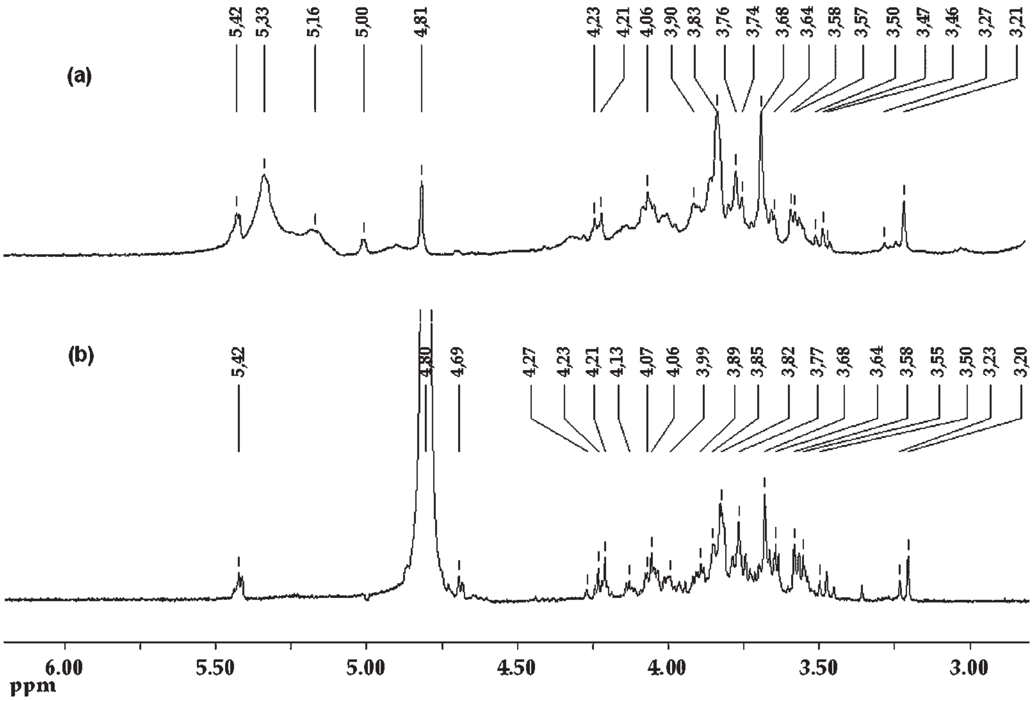

Figure 6 ^1H HR-MAS spectra: (a) melon starch seed (b) watermelon starch seed.

during the VCT, both starches are constituted at least by two domains with different molecular motion, as a consequence of crystal ordination, packing, and amorphous phase dispersion. In spite of a rigid domain to control the relaxation process, the melon starch seed seems to be more rigid and ordinates than the watermelon starch seed. The interpretation of the relaxation values confirm the responses obtained by thermal and X-ray analyses.

Figure 6(a,b) shows the ^{1}H HR-MAS NMR spectra of the melon starch and watermelon starch, respectively. In this technique, to the solid sample is added a drop of deutered water, to find the lock signal and dispersed the sample particles to get a high resolution of hydrogen in the magic angle spinning. The polysaccharide signals at $\sim$ 5.4 ppm, relative to the hydrogen linked to anomeric carbon and a wide signal from 3.6 to 4.6 ppm attributed to the hydrogens linked to CH_2—OH and CH—OH groups, and gluten were detected. Therefore, those spectra clearly show in addition to the water peak at 4.8 ppm a well resolved set of lipid signals (3.2–3.6 ppm) as a minor component, which were detected because its relatively high molecular mobility. The ^{1}H HR-MAS NMR signals of melon [Fig. 6(a)] are broader than the ^{1}H HR-MAS NMR signals of watermelon [Fig. 6(b)], because in the melon the rigid domain controls the relaxation process, as already seen from the proton relaxation data. By contrary, the watermelon ^{1}H HR-MAS NMR signals are narrow confirming the higher molecular mobility of this starch comparing to the melon starch, corroborating the relaxation data information.

CONCLUSIONS

From this work one can conclude that all the techniques used were complementary and allowed us to obtain useful and important information on starch molecular dynamics, which is very much helpful for the food industry. It can be pointed out that proton $T_{1\rho}H$ and ^{1}H HR-MAS are good techniques to evaluate the changes in the morphology and they can be used as a new methodology to explore the starches domain formation.

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