Solid-State NMR to Evaluate the Molecular Changes in the Mango Starch After 8 Years of Storage

André Luis Bonfim Silva Bathista,[1] Emerson Oliveira da Silva,[2] Maria Inês Bruno Tavares,[2] Rogério Junqueira Prad[1]

[1]*Departamento de Física, Instituto de Física—Universidade Federal de Mato Grosso, Mato Grosso, Brasil*
[2]*Laboratório de Alimentos, Instituto de Macromoléculas Professora Eloisa Mano—Universidade Federal do Rio de Janeiro, CT, Bloco J, Ilha do Fundão, Rio de Janeiro, RJ, Brasil, CP 68525*

Received 21 July 2011; accepted 22 December 2011
DOI 10.1002/app.36703
Published online in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Mango seed starch was mainly investigated by 1D solid state nuclear magnetic resonance (NMR) to evaluate the changes in the chemical structure and molecular dynamic, as a response of time storage. X-ray diffraction was used to accompany changes in the morphology after time storage. Thus, the basic work was to understand the changes of mango seed starch, after 8 years storage at room conditions. The solid-state NMR and X-ray diffraction results showed that no changes in both chemical structure and molecular dynamic as well as morphology was found after 8 years of being storage at room conditions. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: mango; seed starch; NMR; relaxation time

INTRODUCTION

The quality of starches and their derivatives is an important factor for their use in food industry. Considering that starches are the major chemical compounds of food such as cereals, they are a subject of many studies. Starches characterization has a great interest in the entire world due to their many applications.[1–5] It is known that each starch presents its own properties, such as composition, crystal type, granule organization and glass temperature, as well as gelatinization process. Thus, the nature of starches infers in their characteristics and consequently uses. Normally, studies on starch focus the identification of crystal type and composition.[1–10] Other studies involve the understanding of chemical structure and molecular dynamics. Analytical techniques, including nuclear magnetic resonance (NMR) via nuclear relaxation time has been well established to be used as a methodology to evaluate heterogeneous samples like starch.[1–3] Fruit seed starch has gained much attention in accordance with their characteristics and benefits for the health. In this work basic solid NMR techniques, such as magic angle spinning (MAS), crosspolarization (CP), crosspolarization magic angle spinning (CPMAS), variable contact time (VCT), and proton spin-lattice relaxation time were chosen to obtain responses on starch chemical composition and morphology, to make a composition-interconnectivity between results.[1–24] The MAS was used with short delay between 90° pulses, to investigate the high mobility domain. The CPMAS was applied to have a response of entire sample. The VCT was employed to evaluated the efficiency of polarization transfer and consequently to identify the mobile domains.

The relaxation parameter gives response on morphology changes that can be interconnected with X-ray results.

The main objective of this work was:

1. Identification of glycosidic carbons by chemical shift assignments employing ^{13}C MAS and CPMAS NMR spectra
2. The study of Anomeric C1–Carbon due to the pharmaceutics needs and food;
3. Domain evaluation due to the variation of amylose and amylopectin composition, employed by a comparison between MAS and CPMAS NMR techniques;
4. Water absorption evaluation through the determination of relaxation times;
5. Chemical resistance to time storage accompanied by changes in the ^{13}C NMR spectra.

EXPERIMENTAL

Material

The starch seed was stored at laboratory conditions for 8 years in a plastic bag. These conditions were chosen to simulate the time of market storage.

Correspondence to: M. B. Tavares (mibt@ima.ufrj.br).

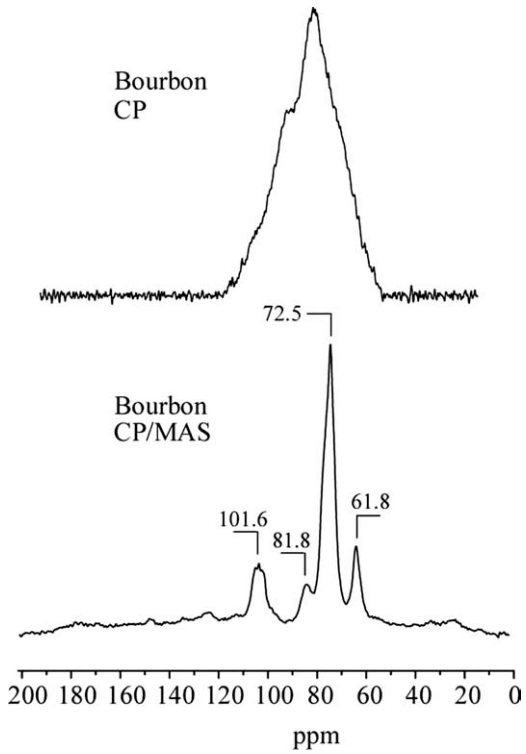

Figure 1 ^{13}C line shape CP and CPMAS.

Solid-state NMR experiments

The solid state ^{13}C NMR spectra were obtained on a VARIAN INOVA 400 spectrometer, operating at 100 MHz for carbon-13. The MAS conditions were, spectral width: 30,000 Hz; acquisition time: 0.04 s; pulse width 90°, recycle delay 0.3 s, and number of transients 5000. For CPMAS, the spectral width: 30,000 Hz; acquisition time: 0.04 s; pulse width 90°, recycle delay 3 s, and number of transients 512. The VCT conditions used were the same for the ^{13}C. A 7-mm static double resonance probe head was used. $\pi/2$ pulse lengths of 3.8 and 4.0 μs were applied for ^{13}C and ^{1}H, respectively. The proton decoupling field strength was $\sim$ 60 kHz, crosspolarization time of 1.0 ms. CPMAS with a range of contact time established as 200–8000 (μs). The processing used was zero filling and line broadening was 50.

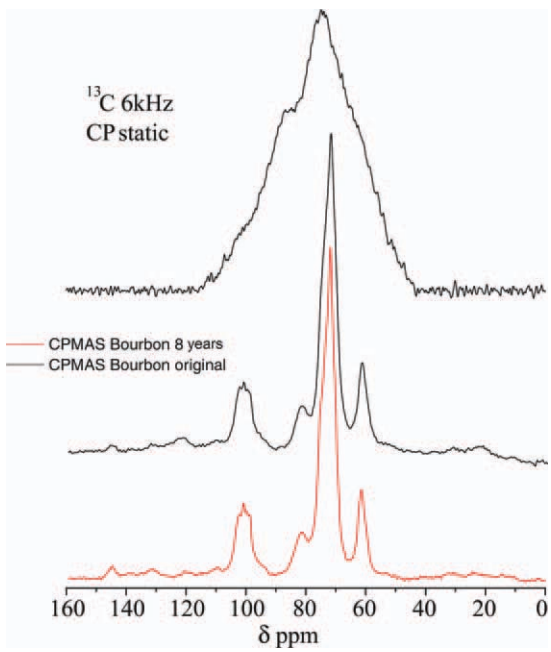

Figure 2 ^{13}C CPMAS spectra mango starch. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Proton spin-lattice measurements conditions

The relaxation time was analyzed in a low field NMR Maran Ultra (Resonance Oxford –UK), using 18-mm NMR tube, operating at 23 MHz for the hydrogen nucleus. The pulse sequence used to obtain data of spin lattice relaxation time was the inversion-recovery (recycle delay—180° – τ – 90° – acquisition data) the 90° pulse of 4.5 μs was calibrated automatically by the instrument software. The amplitude of the FID was sampled for 20 τ data points, ranging from 0.01 to 5000 ms, using 40 data points and 4 scans for each point. The same sample was analyzed at 27°C. The relaxation values and relative intensities were obtained by fitting the exponential data with the aid of the program WINFIT. Distributed exponential fittings as a plot of relaxation amplitude versus relaxation time were performed by using the software WINDXP. Both

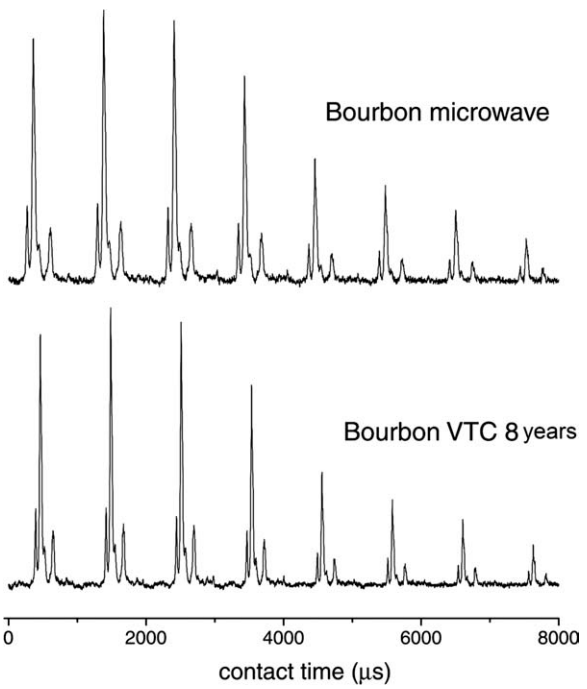

Figure 3 VCT performed for bourbon starch.

TABLE I
$T_{1\rho}^{H}$ Values of Mango Starch Seed Before and After 8 Years of Storage

	Bourbon[a]		Bourbon microwave		Bourbon storage	
Type of carbon	σ (ppm)	$T_{1\rho}^{H}$ (ms)	σ (ppm)	$T_{1\rho}^{H}$ (ms)	σ (ppm)	$T_{1\rho}^{H}$(ms)
C1 anomeric	101.4	4.3	100.9	4.9	100.8	4.3
CH—OH	73.0	4.0	71.6	4.3	71.7	4.2
CH_2—OH	62.4	4.4	61.4	4.7	61.4	4.6

[a] Value from Ref. 9.

WINFIT and WINDXP are commercial programs and come with the low-field NMR spectrometer.

X-ray measurements

X-ray diffraction (XRD) was used to investigate changes in interlayer distance. It was performed using a Rigaku diffractometer with Cu Kα radiation (λ = 0.154 nm, 40 Kv, 120 mA) at room temperature, scanning over the 2θ range from 2° to 50° in 0.05° steps, at a rate of 1° min^{-1}.

RESULTS AND DISCUSSION

^{13}C CPMAS NMR

Figure 1 shows the ^{13}C CP spectra of Bourbon starch with and without MAS. The advantage of spectral resolution with MAS is clearly visible and the peaks correspond to different positions of glucose units and are a typical of starch spectrum. A comparison of the ^{13}C CPMAS spectra with and without rotation for all samples showed the distribution shape of the resolved carbon-13 signals. The ^{13}C CPMAS of mango starch presents signals well defined and located at: 61.8 ppm (CH_2—OH), 72.5 ppm (CH—OH), and 10.6 ppm (C—O—C).

Figure 2 shows a comparison between ^{13}C CPMAS spectra of Bourbon mango starch before and after 8 years of storage. No difference between both spectra was identified. The signal shapes are same and the chemical shift values did not changed, which is a strong indication that no structural changes have occurred in this storage period.

To simulate changes in the starch granule curing the cooking, a treatment employing microwave to cook the starch sample was done to see changes and/or degradation in the chemical structure after

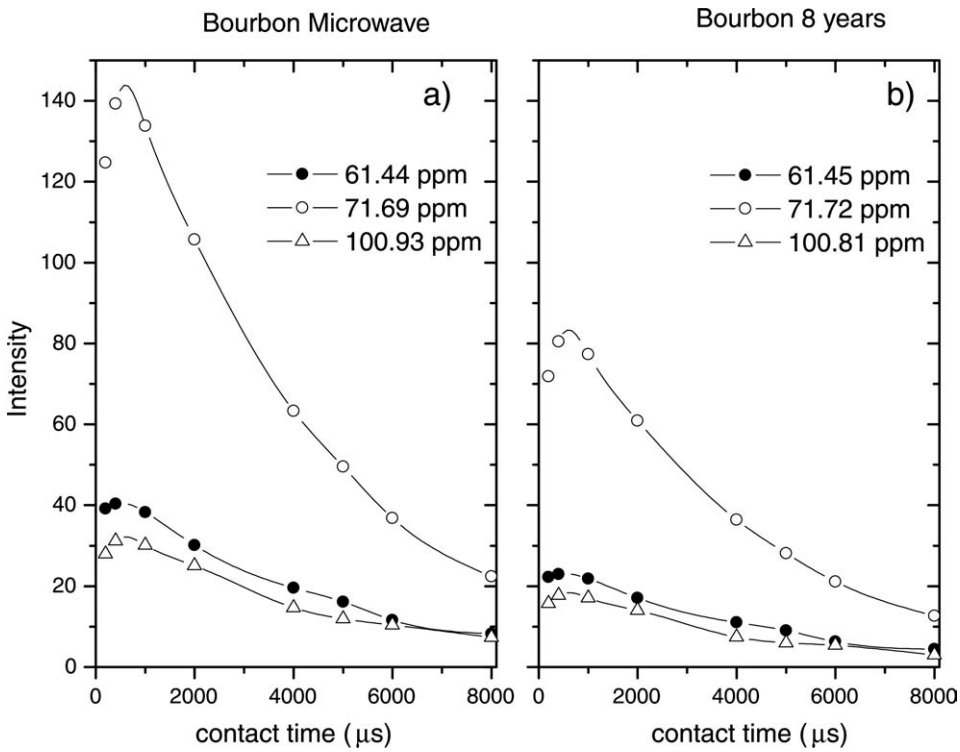

Figure 4 (a) mango treated with microwave, (b) starch storage (8 years).

TABLE II
Values of Proton T_1 for Both Samples

Sample	T_1^H (ms)		
	1Exp	2Exp	
Starch mango	56	5.5	65
Starch storage for 8 year	58	5.5	65
Starch microwave	55	5.8	62

water loss. The motions detected in the range of microseconds to milliseconds (μs–ms) were measured by VCT experiment, which detect the different types of glucose mobility in the range of kHz, according to the intensity of the spectral array (Fig. 3). From VCT experiment no change in the shape decay of resolved carbons for both samples was seen. This is again a strong indication that even the time of storage or microwave cooking did cause structural changes in the mango starch.

From the VCT experiment the $T_{1\rho}^H$ parameter was determined from the resolved carbons, **C**1 anomeric, **C**H—OH and **C**H_2—OH. Table I shows the values of $T_{1\rho}^H$ for the starch mango seed before and after 8 years of storage.

The results shown in Table I agree with the other measurements, confirming that no chemical structural change was detected for starch mango seed after being storage at room temperature for 8 years. Any alteration in the spectra confirms that no microorganism grow up.

Figure 4 shows the decay of individual carbon after VCT experiment. The same decay behavior was observed, which are in accordance with the values of relaxation time determined.

To evaluate the structural changes in the starch seed after being stored for 8 years and after being submitted to microwave, proton spin-lattice relaxation time, measured in a low field NMR, was carried out to evaluate both samples comparing to mango starch. Table II shows the values of proton T_1 for the samples.

From the results listed in Table II no significant change at all was detected in the sample chemical structure or organization after being stored for 8 years at room conditions and also after being cooked in a microwave.

X-ray diffraction was done to both samples to evaluate the crystallinity degree. The crystallinity degree for both samples did not change significantly and the values found were 22.5% ± 0.3% and 21.4% ± 0.6%, for the sample after 8 years of storage and the sample submitted to microwave, respectively. This behavior is in agreement with all results already discussed by NMR.

CONCLUSION

According to the main purpose of this work, the techniques used were able to evaluate the starch fruit seed taking into consideration the changes in the organizational structure or at least in the chemical structure after both types of conditions that the starch seed was submitted. The results showed there are not significant changes in the structure of mango starch, even after 8 years aging and treatment with microwaves.

References

1. Kulik, A. S.; Haverkamp, J. Carbohydr Polym 1997, 34, 49.
2. Tavares, M. I. B.; Bathista, A. L. B. S.; Silva, E. O.; Filho, N. P.; Nogueira, J. S. J Appl Polym Sci 2004, 93, 2151.
3. da Silva, E. O.; Tavares, M. I. B.; Bathista, A. L. B. S.; Priante, N.; Nogueira, J. S. J Appl Polym Sci 2002, 86, 1848.
4. Tang, H.; Hills, B. P. Biomacromolecules 2003, 4, 1269.
5. Tavares, M. I. B.; Bathista, A. L. B. S.; Silva, E. O.; Filho, N. P.; Nogueira, J. S. Carbohydr Polym 2003, 53, 213.
6. Tavares, M. I. B.; Bathista, A. L. B. S.; Silva, E. O.; Filho, N. P.; Nogueira, J. S. In The 2nd Alpine Conference on Solid-State NMR. Chamonix-Mont Blanc—France: International Society of magnetic Resonance and the Groupement Ampere; 2001.
7. Nascimento, A. M. R.; Tavares, M. I. B.; Nascimento, R. S. Int J Polym Mater 2007, 56, 31.
8. Nascimento, A. M. R.; Tavares, M. I. B. Int J Polym Mater 2007, 56, 115.
9. Bathista A. L. B. S.; Tavares, M. I. B.; Silva, E. O.; Costa, P. M.; Nogueira, J. S. Ann Magn Reson 2006, 5, 41.
10. Costa, P. M.; Tavares, M. I. B. Ann Magn Reson 2005, 4, 60.
11. Costa, P. M.; Tavares, M. I. B.; Bathista, A. L. B. S.; Silva, E. O.; Nogueira, J. S. J Appl Polym Sci 2007, 105, 973.
12. Costa, P. M.; Tavares, M. I. B.; Silva, E. O.; Bathista, A. L. B. S.; Nogueira, J. S.; Ferreira, A. G.; Barison, A.; Daolio, C.; Vizzotto, L. Int J Polym Mater 2007, 56, 1135.
13. Maciel, P. D. C.; Tavares, M. I. B. J Appl Polym Sci 2010, 116, 50.
14. Macedo, J. D.; Costa, M. F.; Tavares, M.I.B., et al. Polym Eng Sci 2010, 50, 1466.
15. Iulianelli, G. C. V.; Tavares, M. I. B. Polím Ciência Tecnol 2011, 21, 2.
16. Nogueira, R. F.; Boffo, E. F.; Tavares, M. I. B.; Moreira, L. A.; Tavares, L. A.; Ferreira, A. G. Food Nutr Sci 2011, 2, 350.
17. Komoroski, R. A. High Resolution NMR Spectroscopy of Synthetic Polymers in Bulk; VCH: Deerfield Beach, Florida, 1986, p 118.
18. Bovey, F. A.; Mirau P. A. NMR of Polymers; Academic Press: NY, 1996; p 11.
19. Gil, A. M. The Fifth International Conference on Applications of Magnetic Resonance in Food Science, I, 43. Aveiro, Portugal, 2000.
20. Harris, R. K. The Fifth International Conference on Applications of Magnetic Resonance in Food Science, I, 1. Aveiro, Portugal, 2000.
21. Gidley, M. J.; Bocieck, M. S. J Am Chem Soc 1988, 110, 3820.
22. Gidley, M. J.; Bocieck, M. S.; Gidley, M. J.; Bocieck, M. S. J Am Chem Soc 1985, 107, 7040.
23. Cheetham, N. W. H.; Tao, L. Carbohydr Polym 1998, 36, 285.
24. Conte, P.; Spaccini, R.; Piccolo, A. Prog Nucl Magn Reson Sp 2004, 44, 215.