



ELSEVIER

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Carbohydrate Polymers 53 (2003) 213–216

Carbohydrate
Polymers

www.elsevier.com/locate/carbpol

Short Communication

A molecular dynamic study of the starch obtained from the *Mangifera indica* Cv. *Bourbon* and *Espada* seeds by ^{13}C solid state NMR

Maria Inês B. Tavares*, André Luis B.S. Bathista^a, Emerson O. Silva^b, Nicolau P. Filho^a,
José S. Nogueira^a

^aDF/ICET/UFMT, Cuiabá, Mato Grosso, Brazil

^bDQ/ICET/UFMT, Cuiabá, Mato Grosso, Brazil

Received 8 August 2001; revised 18 March 2002; accepted 16 July 2002

Abstract

The molecular mobility of the starch obtained from *Mangifera indica* Cv. *Bourbon* and *Espada*, has been characterised by ^{13}C solid state nuclear magnetic resonance, using techniques, such as magic angle spinning (MAS) and cross polarisation magic angle spinning (CPMAS) NMR and by proton spin-lattice relaxation time in the rotating frame ($T_1^H\rho$). The CPMAS ^{13}C NMR spectra of these seeds showed three signals for *Bourbon* and two very broad signals for *Espada*. The seeds of the *Mangifera indica* Cv. *Bourbon* and *Espada* are heterogeneous amorphous polysaccharides that present, at least, two domains with distinct molecular mobility. These domains differ in size and chain packing. The variable contact time decay confirms that these starches are amorphous and present one predominant rigid domain. Mango fruits were also analysed by delayed contact time experiments, and the ^{13}C decays showed that the polysaccharides are heterogeneous and contain one rigid domain that controls the relaxation parameter.

© 2003 Elsevier Science Ltd. All rights reserved.

Keywords: Molecular dynamic; Starch; NMR

1. Introduction

Knowledge of the chemical structure and molecular dynamics of the starch present in seeds is necessary to obtain information possible application. The main objective of this work is to obtain useful chemical and physical microstructural information, to understand the molecular behaviour, to contribute to the classification of the seeds in mango fruits, and to evaluate their uses. Solution and solid state NMR spectroscopy were chosen, from among the diversity of techniques that allow us to evaluate the chemical structure, microstructure and dynamics (Bovey & Mirau, 1996; Feng, Feng, & Shen, 1993; Harris, 1996, 2000; Mothé & Tavares, 2000; Silva, Tavares, & Stejskal,

2000; Stejskal, & Memory, 1994; Tavares, Bathista, Silva, & Nogueira, 2000). NMR is important in the evaluation of amorphous and heterogeneous materials, such as starches (Fringant, Rinaudo, Foray, & Bardet, 1998). In particular, the microstructure can be discriminated in solids due to the fact that distinct pulse sequences can be applied to obtain subspectra of components in different domains (Bovey 1996; Harris, 2000; Nogueira & Tavares, 2001; Silva et al., 2000). MAS and CPMAS are examples of techniques that allow us to observe mobile regions and the rigid phase, respectively. For instance, from cross-polarization magic angle spinning with dipolar dephasing (CPMASDD) the mobile regions can automatically be detected together with non hydrogenated carbons (Nogueira & Tavares, 2001). Consequently two distinct phases can be detected. NMR can also provide detailed information on mobility at the molecular level over a wide range of rates, by relaxation time experiment (Feng et al., 1993; Harris, 2000).

* Corresponding author. Tel.: +55-021-2562-7225; fax: +55-021-2270-1035.

E-mail address: mibt@ima.ufrj.br (M.I.B. Tavares).

2. Experimental

2.1. Materials

The starches extracted from the *Mangifera indica* Cv. *Bourboun* and *Espada* seeds were powdered on collection with no further treatment.

2.2. NMR Measurements

All solid spectra were obtained on an INOVA 300 spectrometer, operating at 75.4 MHz for ^{13}C . All solid experiments were carried out at an 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}C NMR spectra were obtained using MAS, with a short recycle time (0.3 s) between 90 degree pulses and 5000 scans; in the CPMAS experiment, the strength of spin-locking field B_1 was 40 kHz; the recycle delay time was 2 s and 3200 scans were used. For the variable contact-time experiment, the lock time ranged from 200 to 8000 μs . Proton $T_{1\rho}$ values were determined from the intensity decay of ^{13}C peaks with increasing contact-times and also from the delayed contact-time experiment, with a spin-lock time varying from 200 to 8000 μs .

3. Results and discussion

The basic MAS ^{13}C was recorded with a short recycle time (0.3 s) between 90° pulses, in order to observe only the mobile species. Both seeds showed sharp signals that were attributed to the oil present in the seeds (Fig. 1). This oil was characterised using solution ^{13}C NMR as a triacylglycerol with long linear chains. It was also observed that the starches do not give signals under these conditions, which is an indication that they do not have domains, which have high molecular mobility on this time scale.

The CPMAS ^{13}C NMR spectra for these seeds are shown on Fig. 2, there are three dominant NMR signals for *Bourboun* and two very broad signals for *Espada* (see Table 1). Other small signals from the oil and weak peaks thought to be due to gluten proteins were also detected. The broad nature of the NMR signals indicated that the seeds of both mango types could be mainly constituted by starch in an amorphous heterogeneous form.

The single exponential decay observed in the variable contact time decay confirms that these samples are due to one large domain, which controls the relaxation process.

After these first results, we decided to make an extraction of the oil presented in the seed starch with acetone for 24 h. The starch, after removal of the acetone, was dried and analysed by the same techniques. The MAS ^{13}C recorded under the conditions specified in the experimental section, did not show any signals at all.

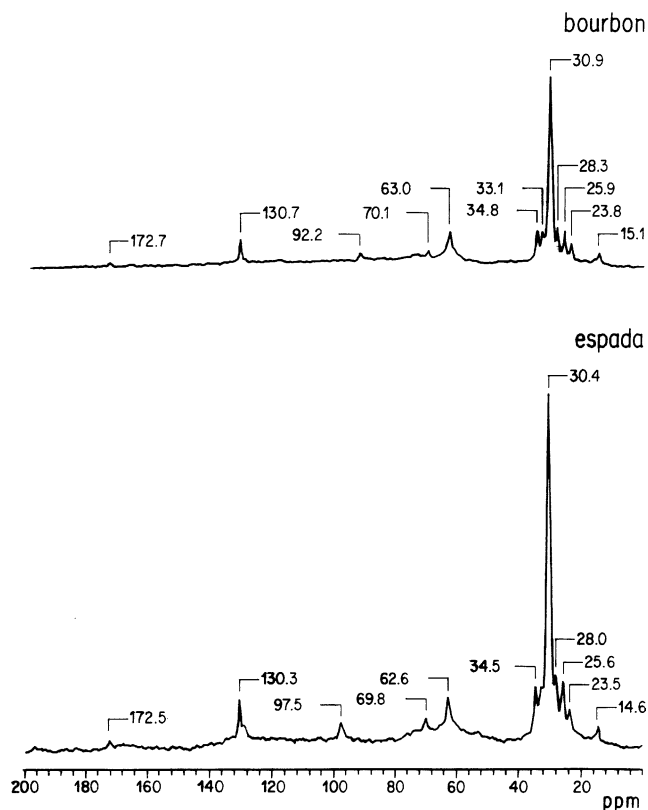


Fig. 1. MAS ^{13}C , recorded with short delay time (0.3 s) between 90° pulses, for both mango seeds.

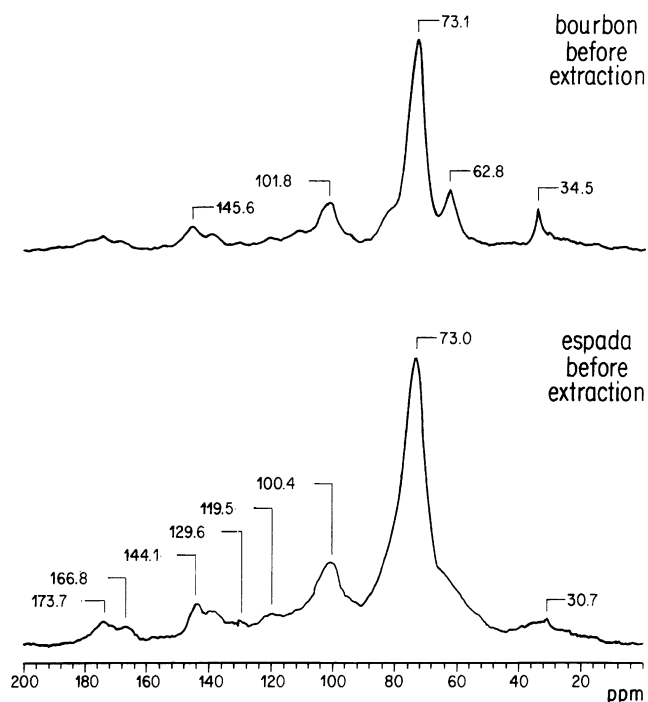


Fig. 2. CPMAS ^{13}C NMR spectra of *Bourboun* and *Espada* seeds, before acetone extraction.

Table 1
Chemical shift, measured for both types of mango seeds before extraction

Type of carbon	<i>Bourboun</i> $\delta(\text{ppm})$	<i>Espada</i> $\delta(\text{ppm})$
C1 anomeric	101.8	100.4
CH–OH	73.1	73.0
CH ₂ –OH	62.8	–

The CPMAS ¹³C spectra (Fig. 3) showed three wide NMR signals for *Bourboun* and two very broad signals for *Espada*. These NMR lines derive from the polysaccharide. The difference in the CPMAS line broadening lead us to suggest that these starches have differences in the quantity of chemical components. According to these observations *espada* starch seems to be more amorphous than bourbon starch.

The $T_1^H\rho$ parameter, for both seed starches, was obtained from the variable contact time (VCT) experiment. $T_1^H\rho$ depends on the extent of dipolar interaction which is dominated by near neighbour spin diffusion. $T_1^H\rho$ was also obtained from delayed contact time (DCT) before and after extraction. The values for the $T_1^H\rho$ parameter measured by VCT are listed in Tables 2 and 3. The $T_1^H\rho$ values measured by DCT, in this experiment the contact time is constant and the values of $T_1^H\rho$ detected come from the mobile region are listed in Tables 4 and 5.

The values of $T_1^H\rho$, obtained by VCT, for bourbon indicate that the starches are non-ordered either within

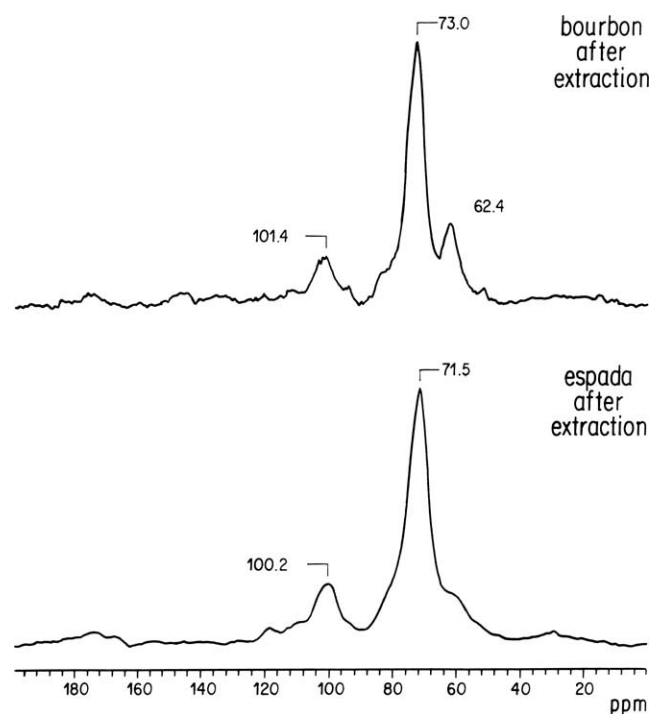


Fig. 3. The CPMAS ¹³C spectra for *Bourboun* and *Espada* seeds, after acetone extraction.

Table 2
 $T_1^H\rho$ values, as a function of chemical shift, obtained by VCT before extraction

Type of carbon	<i>Bourboun</i>		<i>Espada</i>	
	$\delta(\text{ppm})$	$T_1^H\rho(\text{ms})$	$\delta(\text{ppm})$	$T_1^H\rho(\text{ms})$
C1 anomeric	101.8	2.6	100.4	4.7
CH–OH	73.1	5.3	73.0	5.3
CH ₂ –OH	62.8	7.3	–	–

Table 3
 $T_1^H\rho$ values, as a function of chemical shift, obtained by VCT after extraction

Type of carbon	<i>Bourboun</i>		<i>Espada</i>	
	$\delta(\text{ppm})$	$T_1^H\rho(\text{ms})$	$\delta(\text{ppm})$	$T_1^H\rho(\text{ms})$
C1 anomeric	101.4	1.9	100.2	1.8
Ch–OH	73.0	5.1	71.5	6.1
CH ₂ –OH	62.4	7.1	–	–

the time scale of the dipole–dipole interaction or in the sense that the average distance between dipoles is too great for efficient transfer.

The $T_1^H\rho$ values, obtained by DCT, for both mango seeds, show no significant difference. The distribution of starch components now seems to be homogeneous. These values should represent the more mobile fractions and may represent the α (1–6) branching of the amylopectin chain. These branches exhibit higher relaxation rates implying fast molecular reorientation in the branches, causing higher frequency molecular motions.

Table 4
 $T_1^H\rho$ values, as a function of chemical shift, obtained by DCT before extraction

Type of carbon	<i>Bourboun</i>		<i>Espada</i>	
	$\delta(\text{ppm})$	$T_1^H\rho(\text{ms})$	$\delta(\text{ppm})$	$T_1^H\rho(\text{ms})$
C1 anomeric	101.8	4.6	100.4	3.7
Ch–OH	73.1	4.0	73.0	3.3
CH ₂ –OH	62.8	4.3	–	–

Table 5
 $T_1^H\rho$ values, as a function of chemical shift, obtained by DCT after extraction

Type of carbon	<i>Bourboun</i>		<i>Espada</i>	
	$\delta(\text{ppm})$	$T_1^H\rho(\text{ms})$	$\delta(\text{ppm})$	$T_1^H\rho(\text{ms})$
C1 anomeric	101.4	4.3	100.2	3.6
Ch–OH	73.0	4.0	71.5	3.8
CH ₂ –OH	62.4	4.3	–	–

Acknowledgements

The authors would like to thank PETROBRAS/CENPES/Gerência de Química for the use of solid state spectrometer and to CNPq, FAPEMAT, BASA and PRONEX-CNPq 0327.00/00 for financial support.

References

- Bovey, F. A., & Mirau, P. A. (1996). *NMR of polymers*. New York: Academic Press.
- Feng, H., Feng, Z., & Shen, L. (1993). *Polymer*, 34, 2516.
- Fringant, C., Rinaudo, M., Foray, M. F., & Bardet, M. (1998). *Carbohydrate Polymers*, 35, 97.
- Harris, R. K. (1996). NMR studies of solid polymer. In A. H. Fawcett (Ed.), *Polymer spectroscopy*. England: Wiley.
- Harris, R. K. (2000). Recent advances in solid state NMR. *Proceedings of the fifth international conference on applications of magnetic resonance in food science* (pp. 18–20). Portugal: University of Aveiro.
- Mothé, C. G., & Tavares, M. I. B. (2000). Anais do. *The Fifth International Conference on Applications of Magnetic Resonance in Food Science, Aveiro, Portugal, 1*, 140.
- Nogueira, R. F., & Tavares, M. I. B. (2001). *Journal of Applied Polymer Science*, 81, 261.
- Silva, N. M., Tavares, M. I. B., & Stejskal, E. O. (2000). *Macromolecules*, 33, 115.
- Stejskal, E. O., & Memory, J. D. (1994). *High resolution NMR in the solid state*. New York: Oxford University Press.
- Tavares, M. I. B., Bathista, A. L. B. S., Silva, E. O., & Nogueira, J. S. (2000). Annals of *The Fifth International Conference on Applications of Magnetic Resonance in Food Science, Aveiro, Portugal, 1*, 138.