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Short communication

Fatty acid composition of *Jatropha curcas* seeds under different agronomical conditions by means of ^{1}H HR-MAS NMR

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ABSTRACT

This work evaluated the influence of drought and nitrogen fertilization on the fatty acid composition of *Jatropha curcas* seeds by means of ^{1}H HR-MAS NMR, a tool which allows obtaining chemical information directly from the seeds. It was found that only the drought conditions led to the decrease of polyunsaturated fatty acid contents and to the increase of those monounsaturated. No changes were observed for the contents of saturated fatty acids. The lipid profile reached without irrigation may be considered the most effective for improving biodiesel properties, such as cetane number and oxidative stability. At last, there was no influence on the fatty acid composition with different levels of nitrogen fertilizer application.

1. Introduction

Biodiesel is composed of methyl and ethyl esters containing a long fatty acyl chain produced through transesterification reactions of triacylglycerols present in feedstock such as vegetable oils, animal fats, waste cooking oil and sideproducts from vegetable oils production [1,2]. Biodiesel is the main alternative biofuel to replace fossil fuels in the world, since their physical-chemistry properties are very similar to those of petrodiesel. This enables its use either, neat or blended with petrodiesel without demanding any modification on diesel engines. Moreover, it presents some important advantages regarding environmental point of view, such as biodegradability and reduction of exhaust emissions with almost no sulfur and aromatic polycyclic compounds [3–6].

Since biodiesel is obtained from vegetable oils, it is crucial to find resources able to provide the sufficient feedstock to its production. In this context, inedible oils from plants can provide a good alternative once there is no competition with food crops. In this way, *Jatropha curcas* L. (Euphorbiaceae) known as physic nut is an important inedible plants widely distributed in Central and South America, Africa and South-East Asia [7]. This specie is considered a promising energy source, popularized as a unique candidate among renewable energy sources due to its peculiar features like drought tolerance, rapid growth, easy propagation, high seed production as well as high oil content [6,8]. The oil mass fraction ranges from 30 to 50% by fruit and the kernels from 45 to 60%, with an average production of 1.6 Mg ha^{-1} [9,10]. *J. curcas* is the second highest oil-yielding crop in Brazil, just behind palm oil [11]. Beside crop productivity, the oil content and fatty acid composition are the main issues that should be addressed in order to select the best agronomic conditions that may lead to advantageous vegetable oils features for the biodiesel production [12]. In spite of the importance of *J. curcas*, there are only few works in the literature regarding the influence agriculture practices in the composition and content of its vegetable oils.

In view of all this information, the present work reports the influence of irrigation and nitrogen administration on the chemical composition of vegetable oil of *J. curcas* seeds by means of ^{1}H High

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Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy. Such tool allows the extraction of chemical information directly from seed in their natural state without significant pretreatment [13].

2. Materials and methods

2.1. Jatropha curcas cultive and seeds obtention

Jatropha curcas plants were grown along three years (2011–2014) at the experimental station of the Department of Biosystems Engineering, University of São Paulo, Piracicaba, São Paulo, Brazil (latitude 22°41′58″ S, longitude 47°38′42″ W and 530 m high). The regional climate is classified as tropical, according to the classification of Köppen-Geiger [14]. The average annual rainfall is 1328 mm and the annual temperature ranges from 10 to 30 °C [15]. Plants were cultivated under irrigation by central pivot (I) and non-irrigated (NI) under different levels of nitrogen (0, 50, 100 and 150%), according to nitrogen fertilizer recommendation [16]. The soil used for *J. curcas* cultivation presented an initial average nitrogen level of 1.0 Kg^{-1}. Nitrogen fertilization was performed by three application of urea over the projection of the tree topping on the ground. A total of 2900 mm rainfall has occurred over the experiment time, while the irrigated plants received an increment of 1248 mm irrigation depth applied, resulting in a total of 4148 mm of water over three years. Respective amounts of rainfall and irrigation were of 1117 and 287 mm water, in the first year, 1195 and 230 mm in second year and 588 and 731 mm water in third year of cultivation.

Mature fruits of *Jatropha curcas* were collected at harvest time from the third year of cultivation (December 2013 to March 2014). Follow the fruits were dried at room temperature in shadow. The seeds were separated from the fruit and cleaned manually to remove all foreign material. Follow, the seeds were dried under 60 °C and humidity conditions until reaching in constant weight. Each treatment was represented by three independent replicates of 10 seeds (n = 30).

2.2. ^{1}H HR-MAS NMR analysis

In order to be possible the NMR analysis through HR-MAS NMR tool, the seeds without peel, which were manually removed, were powdered in a mortar and then 5.0 mg were inserted in a semi-spherical zirconium rotor followed by addition of 40 mm^3 of $CDCl_3$, containing 0.05% of TMS. All NMR experiments were acquired at 293 K on a Bruker AVANCE NMR spectrometer operating at 9.4 T, observing ^{1}H at 400.13 MHz, equipped with a four channel (^{1}H, ^{13}C, ^{15}N and D) 4-mm HR-MAS probe. The rotors were spun at 5 kHz on the magic angle (54.74°). ^{1}H HR-MAS NMR spectra were acquired in triplicate with 64 K data points distributed on a spectra width of 6510 Hz, providing a digital resolution of 0.12 Hz by applying 90° excitation pulses, 16 scans, relaxation delay of 20 s. All these parameters give a quantitative issue of the NMR analysis. The total experiment time was 10 min for each sample (n = 3), including sample preparation and NMR spectra acquisition. Before each ^{1}H HR-MAS NMR spectrum was acquired, the probe was tuned and matched, and the shimming was optimized. In addition, the magic angle was daily tuned. The spectra were processed by applying an exponential multiplication of the FIDs by a factor of 0.3 Hz prior to Fourier transform with zero-filling to 128 K. The spectra were referenced to the TMS signal at δ 0.00.

2.3. Statistical analysis

Analysis of variance (F test) was applied to evaluate if there are significant differences among the treatments [17]. Test of normality was performed with PROC UNIVARIAT. Comparisons between means were carried out at a p value of 0.05 and using Tukey's test.

3. Results and discussion

The ^{1}H HR-MAS NMR spectra acquired directly from the seeds of *J. curcas* showed typical signals from triacylglycerols (Fig. 1). The fatty acid composition was similar to those ones reported in the literature for several vegetable oils with well-known chemical shifts. The signal notation on the ^{1}H NMR spectra follows the ascending order of chemical shift. Thus starting with the signal A (δ 0.88), which corresponds to methyl groups of the acyl moieties from all fatty acids (FA) (except for linolenic acid) and finishes with signals I of the olefins of the unsaturated FA (δ 5.36). Apart from the signals already mentioned, the ^{1}H NMR spectra exhibited signals at δ 4.14–4.30 and δ 5.27 from the presence of two alpha methylenes (α,α′-CH_2: G) and beta methine group (β-CH: H), respectively, both relating to the glycerol backbone. Additionally, it was observed signals at δ 1.25–1.30, which are typical of methylene groups of all FA acyl chains (B), δ 1.62, which corresponds to β carbonyl hydrogens from all FA (C), δ 2.03 characteristic of methylene groups alpha olefins (i.e. allylic hydrogens) of all unsaturated FA (D), δ 2.31 revealing the presence of alpha carbonyl hydrogens from all FA (E) and finally at δ 2.77 corresponding to methylene hydrogens between two olefins (i.e. diallylic hydrogens) from all polyunsaturated FA (F) (Fig. 1). In summary, all the aforementioned signals refer to fatty acid acyl chains esterified to glycerol backbone.

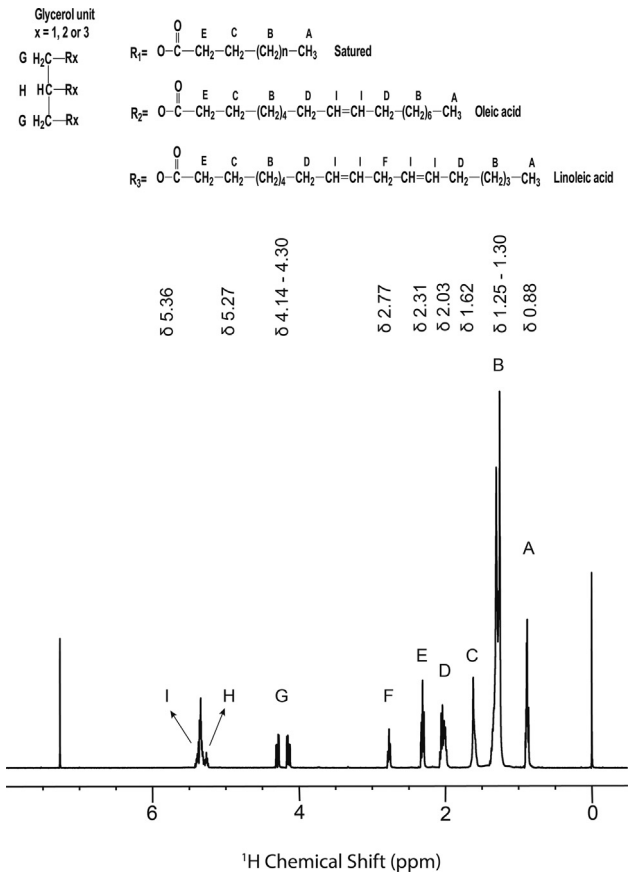

Fig. 1. A typical ^{1}H HR-MAS NMR spectrum acquired directly from *J. curcas* seeds with respective signal assignments of the glycerol unit and the different fatty acids. The figure also shows the structure and signal notation used for glycerol and fatty acid hydrogen nuclei.