



Analysis of Oil Content in Jatropha by Nuclear Magnetic Resonance Spectrometry

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Thirty two diverse Jatropha curcas L. cultivars were analyzed for their oil content by the standard Soxhlet extraction method using hexane as solvent. The results were then compared with those obtained by nuclear magnetic resonance (NMR) spectrometer. The cultivars had a wide range in oil content, which ranged from 4.8 to 38.8% by the Soxhlet method; and from 6.0 to 38.9% by the NMR method. The values

of oil content determined by the NMR method were highly significantly correlated ($R^2 = 0.9929$, p<0.0001, n=32) with those obtained using the Soxhlet method. The NMR method is simple, non-destructive, rapid and accurate for the routine analysis of oil content in Jatropha.

Keywords Routine nondestructive method, *Jatropha curcas*, NMR spectrometer, oil content

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Introduction

Nuclear Magnetic Resonance (NMR) Spectrometry has been used for determining oil content in seeds of various oil seed crops. The technique has been calibrated for several oil seed crops including groundnut, soybean and canola; and moreover, the technique can be calibrated for other species as needed (Madsen 1976; Jambunathan et al. 1985; Rubel 1994; Kaushik et al. 2007).

The use of the pulsed NMR technique has also been standardized to simultaneously determine oil and moisture content in small- as well large-seeded crops such as rape seed, soybean, maize or groundnut (Gambhir and Agarwala 1985; Jambunathan et al. 1985; Rubel 1994). In brief, NMR spectrometry provides a rapid, accurate and non-destructive estimation of oil content in whole intact seeds of a range of crops; and the technique is most suited for large volume routine analysis for oil content in crops as needed in conventional and molecular breeding research.

The aim of this communication is to describe the results of a study conducted for the comparative evaluation of the NMR spectrometry and the Soxhlet extraction methods for determining oil content in *Jatropha curcas* L. seeds. *Jatropha* is emerging as a potential crop in meeting the challenge of shortage of non-edible oil for biodiesel (Achten et al. 2010; Behera et al. 2010; Brittaine 2008; Koh and Ghazi 2011). The research on Jatropha has also shown that there is a large variability in different accessions of *Jatropha* from diverse agroclimatic regions (Kaushik et al. 2007; Divakara et al. 2010).

Moreover, recent research at the ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) showed that *Jatropha* has the potential to grow on relatively degraded, low fertility lands; and has the capacity to rehabilitate the degraded lands by adding large amounts of carbon through litter and leaf fall, and pruned biomass (Wani et al. 2012).

Materials and Methods

Jatropha seeds were received in our laboratory from plant breeders involved in research on Jatropha research at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, for determining oil content. This provided us the opportunity to calibrate the NMR method for determining oil contents in Jatropha cultivars/accessions using Soxhlet extraction as the standard method. The results obtained by the NMR method were evaluated by comparing them with those obtained by the Soxhlet extraction method using hexane as the solvent.

While intact seeds of *Jatropha* cultivars were used for the analysis of their oil contents by the NMR method; the Jatropha seeds were ground in a Krups, KM 75 (Robert Krups, 5650 Solingen, Germany) blender; and the Jatropha meal was extracted with hexane for 18 h in a Soxhlet apparatus. Oil content was adjusted to 5% moisture content. For the NMR method, Bench top NMR (MQA-6005, Oxford Instruments, UK) spectrometer was used. Detailed description of the NMR and Soxhlet methods used for determining oil content in Jatropha cultivars are described in Jambunathan et al. (1985).

The oil content by the NMR and Soxhlet methods was determined using two replications (two independent analyses) and the values reported are the means of two replications. The values of oil content reported are adjusted to 5% moisture content.

Simple correlation between the values of oil content determined by the NMR and Soxhlet methods was obtained by regression analysis.

Results and Discussion

The oil content in 32 *Jatropha* cultivars analyzed using the Soxhlet and NMR methods are given in Table 1. The results showed that the *Jatropha* cultivars used in the study captured a wide diversity in oil contents. For example, the oil content analyzed using the Soxhlet extraction method varied from 4.8 to 38.8% with a mean value of 22.23%. The oil content in the *Jatropha* cultivars analyzed by the NMR method ranged from 6.0 to 38.9% with a mean value of 22.99%.

The results on the precision in determining oil content by the Soxhlet and NMR methods as judged by the range, mean and standard deviation (SD) and standard error (SE) showed that the two methods had acceptable precision in the analysis of oil content in *Jatropha* cultivars (Table 2). However, the precision by the NMR method seemed better with relatively lower values of SD and SE in the determination of oil content.

Regression analysis of the results on oil content of 32 *Jatropha* cultivars showed that the values obtained by the NMR method were highly significantly correlated with those obtained by the Soxhlet extraction method. The regression equation showing the relationships between the two methods is represented by the following equation:

Oil by Soxhlet method =
$$1.0659$$
 (Oil by NMR method) -2.0786 , $R^2 = 0.9929$ (P< 0.0001 , n = 32) (1)

As mentioned in the Introduction, the NMR method is a very versatile method and can be adopted for the analysis of oil and moisture contents in a range of crops with a wide range in oil contents. Our results on the analysis of oil contents in diverse Jatropha cultivars indeed establish that the NMR is a convenient, rapid and accurate and non-destructive method for routine analysis of oil content in non-edible seed crop like Jatropha. This should aid breeders in the selection and breeding of *Jatropha* for enhanced oil content (Kaushik et al. 2007; Divakara et al. 2010).

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Table 1. The oil content in 32 *Jatropha* cultivars/accessions determined by the Soxhlet extraction and NMR methods

Sample No.	Oil content (%)		
	Soxhlet method	NMR method	
1	23.5	25.5	
2	16.7	18.0	
3	15.1	16.8	
4	15.5	16.0	
5	11.2	12.9	
6	36.8	36.1	
7	13.8	15.5	
8	13.6	15.8	
9	37.0	36.2	
10	30.3	30.9	
11	32.3	33.7	
12	38.8	37.0	
13	15.5	16.9	
14	11.9	13.6	
15	9.1	10.7	
16	35.2	36.2	
17	14.7	13.6	
18	35.9	36.6	
19	11.4	13.0	
20	19.3	20.6	
21	14.9	16.2	
22	18.2	18.9	
23	36.9	36.3	
24	37.2	38.9	
25	13.2	14.4	
26	10.1	11.4	
27	30.0	31.2	
28	13.8	14.5	
29	19.4	20.1	
30	4.8	6.0	
31	36.7	35.3	
32	38.4	37.0	

Table 2. Precision in the analysis of soil content in *Jatropha* seeds by Soxhlet and NMR methods

	Oil content (%)	
	Soxhlet method	NMR method
Range	4.8 - 38.8	6.0 - 38.9
Mean	22.23	22.99
SD	11.04	10.42
SE	1.952	1.841