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A simplified approach to transesterification for GC-MS analysis in *Jatropha curcas*

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ABSTRACT

The development of methods to analyze fatty acids which require solvent extraction, purification, hydrolysis and derivatization procedures, and which are not lengthy or cumbersome, are in great demand. Jatropha curcas, being non edible and possessing high seed oil content, has been gaining importance as a biodiesel, besides several medicinal applications. Fatty Acid Methyl Esters (FAMEs) preparation is the critical step in biofuel production. In this study, we report simple and efficient methods for FAMEs preparations to screen large number of samples of J. curcas with respect to fatty acid compositions. These procedures are based on methylation of fatty acids using commercial anhydrous HCl/ acetyl chloride and methanol. GC-MS of FAMEs was done and peak percentage area for the most prevalent monounsaturated Fatty Acids i.e. Oleic acid, were found to be 48.61±0.06 by Method 1 and 48.20±0.08 by Method 2. Major polyunsaturated fatty acids accounted for about 26.04±0.23 and 24.07±0.4 6 percent area respectively for the methods 1 and 2. The FAMEs yields from both methods are comparable, although the procedure used in Method 2 is faster. These values, being in correct range, indicate that these single step extraction procedures are efficient, time saving as well as economical for the derivatization process. These two methods used for studying the fatty acids composition in the seeds can be useful for screening of large number samples and can also facilitate population studies.

Keywords: *Jatropha*, Fatty Acid Methyl Esters (FAMEs), Transesterification, Biodiesel

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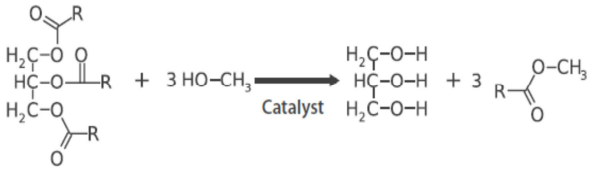
INTRODUCTION

At present, the world's energy requirements are mostly met through non-renewable resources like petrochemicals, naturals gas, coal, etc. If demand of petroleum based fuel continues to rise at the same rate, these depleting resources may not be available in near future at reasonable cost. It is, therefore, time that alternative sources of fuel energy are explored. A substitute fuel must be technically feasible, economically competitive, environmentally acceptable and easily available [1-4]. Fatty Acid Methyl Esters (FAMEs) derived from vegetable oils have been gaining importance as an alternative fuel for diesel engines. Biodiesel can be used either in pure form or as blend of conventional petro-diesel in automobiles without any major engine modifications [5-9]. There are various non-edible and edible oils which can be used as alternative source for engine fuel. However, use of non-edible oil is preferable since they are not suitable as a food source. *Jatropha curcas* L. is one such non-edible oil belonging to the *Euphorbiaceae* family. It is a fast growing, non-edible, subtropical tree that produces oil bearing seeds from which a wide variety of bio-based materials including biodiesel, biojet fuel, biodegradable hydraulic fluids, and various organic chemicals can be obtained. Because of its various economic and sustainable characteristics, it has been identified as one of the most viable feedstocks for extensive production of sustainable vegetable oil, biomass and protein. Economic

relevance of *J. curcas* for biodiesel production has endorsed world-wide prospect of its germplasm for crop improvement and breeding. However, the main challenge being faced by Breeders is development of superior quality oil varieties with high seed and oil yields. While selecting better quality oils in terms of fatty acid profile, a large number of plant samples are needed to be screened. This requires phenotyping for fatty acid profile of the seeds of large number of plants. It also requires a standardized laboratory technique that gives accurate results in less time, a clear picture of types (saturated and unsaturated) of fatty acids present and should also help in determining nutritional quality and shelf life of oil. For fatty acid analysis, Gas chromatography is a rapid, cost effective method with high reducibility [10]. However, before fatty acid analysis by Gas Chromatography , they are required to be converted into their volatile forms- methyl esters, which is a tedious and time consuming process, particularly when dealing with large number of samples. Keeping this in consideration, we have modified some classical FAME preparations methods and standardized it for our plant system i.e. *Jatropha curcas*.

MATERIALS AND METHODS

Plant material: *Jatropha* seeds were borrowed from NBPGR, New Delhi. A total of 5 g of seed was taken as sample and ground to powder for fatty acid methyl esters preparation.



A chemical reaction illustrating Fatty Acids Methyl Esters formation from a Triglyceride

Method 1. A sample containing 0.5 g of powdered seeds was introduced at the bottom of a screw-capped tube. To this, 2 ml of methanolic Hydrochloric acid, 2 ml of methanol and 0.5 ml of hexane were added. The tube was firmly closed and heated at 100°C for one hour (tube was shaken several times during the process of heating). After an hour, the tube was removed from steam water bath and cooled down. After cooling, 1 ml of hexane and 1 ml of water were added. Hexane layer was separated carefully after a short centrifugation. The experiment was carried out in replications.

Method 2. Samples were put in test tubes and to each tubes 4 ml of the methylation mixture which consisted of methanol/acetyl chloride, 20:1 v/v, followed by 1 ml hexane was added to the tubes. The samples were then heated at 100°C for 15 minutes. A single methanol/hexane phase was observed in tubes when tubes were removed from steam bath chamber. After cooling to room temperature, 1 ml distilled water was added. As the water was added to mixture, two phases were formed very quickly. The mixture was centrifuged, the upper (hexanic) layer extracted and placed into the chromatograph vial for injection.

GC-MS Analysis: The hexanic extracts of *Jatropha* containing FAMEs which were derived using Method 1 and Method 2 were subjected to GC-MS analysis. The samples were injected into a Gas Chromatograph (Shimadzu). The Gas Chromatograph had a capillary column of length = 30m, inner diameter =0.25mm and film thickness = 0.25µm. The injector column temperature was maintained at 250°C and the oven temperature was programmed linearly. Injection mode was split type at 250°C; total and column flow were 30.50 ml/min and 2.50 ml/min respectively at a linear velocity of 58.30/sec., purge flow of 3.0 ml/min, ion source temperature 200°C and split ratio of 10. The identification of the peaks was achieved by retention times by comparing them with authentic standards analyzed under the same conditions.

RESULTS AND DISCUSSION

The Gas Chromatography Mass Spectroscopy results are shown in Figures 1 and 2. A total of seven major fatty acids presented in Table 1 have been identified from the total ion chromatogram of the GCMS. All the values are derived in area percentage from the respected peak of chromatogram. The study showed that the ratio of the total unsaturated to saturated fatty acids is approximately 3:1 for both the methods. This means that *J. curcas* seed oil contains more unsaturated fatty acids [11] .

Fig 1: Gas Chromatogram (GCMS) for composition of fatty acids in *Jatropha* seed by method 1.

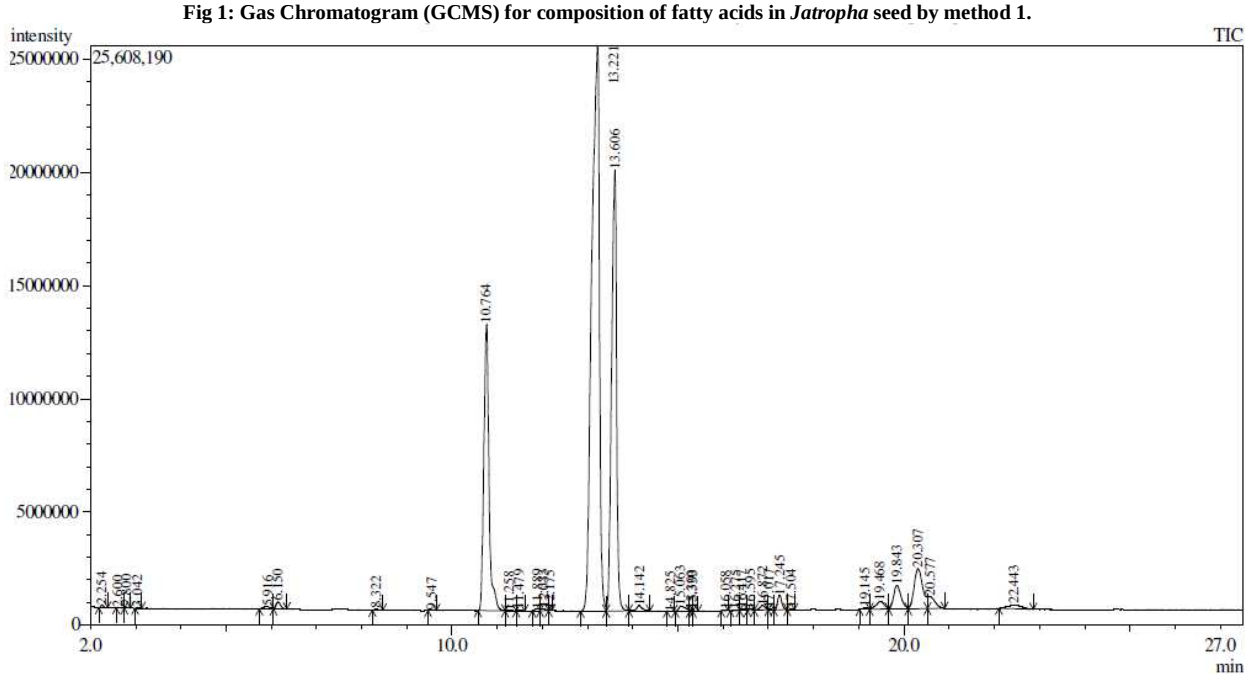
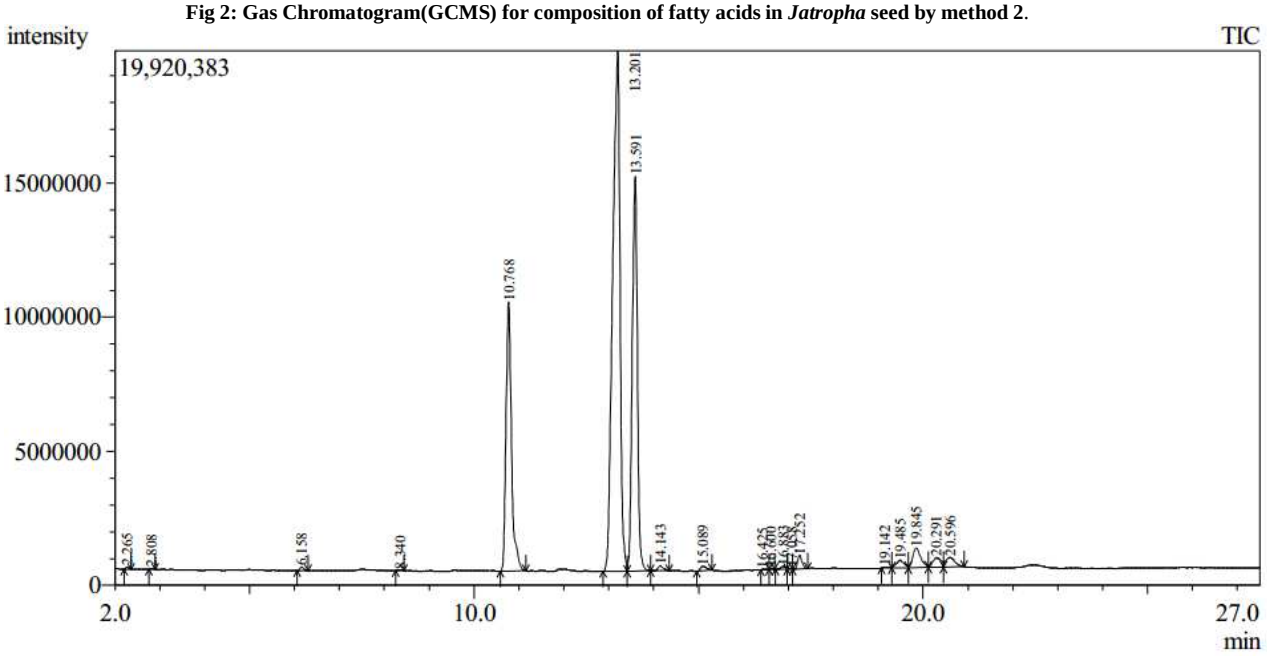


Fig 2: Gas Chromatogram(GCMS) for composition of fatty acids in *Jatropha* seed by method 2.



Major monounsaturated fatty acids accounted for about 50.90±0.26 for method 1 and 49.74±0.56 for method 2. The high value of monounsaturated fatty acids in *Jatropha curcas* contributed to its biodiesel characteristics. The most abundant monounsaturated fatty acid, Oleate, which is known to have excellent biodiesel characteristics with respect to ignition quality, nitrogen oxides (NO_x) emissions and fuel stability [12], has shown 48.61±0.06 and 48.20±0.08 percentage yield respectively by two methods.

Table 1. Fatty acids profile of *Jatropha curcas* seed obtained from the GCMS

		Method 1		Method 2	
Fatty acids	**Molecular weight**	**Retention time (Min)**	**Percentage Yield**	**Retention Time (Min)**	**Percentage Yield**
Oleic acid (18:1 n−9)	296	13.20±0.02	48.61±0.06	13.25±0.03	48.20±0.08
Linoleic acid (18:2 n-6)	294	13.59±0.03	25.63±0.63	13.62±0.01	24.30±0.52
α-Linolenic acid 18:3 (n-6)	292	14.16±0.03	0.34±0.01	14.14±0.02	0.31±0.01
Palmitic acid (C16:0)	270	10.76±0.01	18.69±0.44	10.75±0.03	16.42±0.49
stearic acid (C18:0)	330	19.48±.0.01	0.74±0.02	19.46±.0.01	0.70±.0.01
cis-Vaccenic acid (18:1 n−7)	282	20.29±.0.002	1.99±0.02	19.84±0.02	1.95±0.02
Arachid acid (C20:0)	326	15.10±0.04	0.34±.0.005	15.063±0.02	0.39±.0.005
	Total SFAs		**19.80±0.34**		**17.54±0.33**
	Total MUFAs		**50.90±0.26**		**49.74±0.56**
	Total PUFAs		**26.04±0.23**		**24.07±0.46**

Each data is mean of three replicates ± standard deviation (S.D). SFA- Saturated Fatty acids, MUFAs - Monounsaturated Fatty acids, PUFA- Polyunsaturated Fatty acids

The major polyunsaturated fatty acids accounted for 26.04±0.23 and 24.07±0.46 of total fatty acids respectively for the methods 1 and 2 respectively. Following Oleic acids, the major fatty acid found in *Jatropha* seed oil was linoleic acid with 25.63±0.63 and 24.30±0.52 yield by the methods 1 and 2 respectively. It has significant importance in surface coating industries and biolubricant base oil applications [13]. Yield of total saturated acids were found to be 19.80±0.34 by method 1 and 17.54±0.33 by method 2. However, most abundant saturated fatty acid, palmitic acid, was observed to be comparable in yield as found in method 1and 2. Other major fatty acids include, stearic acid, linolenic acid , vaccenic and arachidic acids as mentioned in Table1. Few other fatty acids which were present in minute quantities are as elucidated in Figures 1 and 2.

To meet the fuel needs of an ever-growing global population, researches need to develop high yielding stable varieties. Biodiesel is a fuel generally consisting of a mixture of FAMEs which is used as an alternative fuel in pure form or in combination with petroleum diesel for its environmental benefits. Biodiesel is conveniently manufactured from vegetable oils by transesterification of triglycerides with methanol. However, for a diverse plant, like *Jatropha curcas*, extraction of seed oil and then conversion to FAMEs from a large number of plant samples for screening purpose, is a tedious process. The fatty acids preparation methods attempted in this study have shown recovery of total fatty acids being at par with traditional method [14]. The traditional method, being a multiple step procedure, increases the possibility of contamination, in addition to sample losses. In comparison, these two modified methods are easy and also have reduced steps. Fatty acid yield percentage is nearly same in both the methods, although method 2 is faster. But in method 2, care should be taken while using acetyl chloride which has tendency to bump out on boiling. These direct methylation methods merge extraction and transesterification into a single step, thus giving rise to a simpler and faster analysis and also consume less solvent [15]. These FAMEs preparation methods result in a better extraction of the total fatty acid methyl esters from seed sample of *Jatropha curcas* without prejudice or selectivity towards any lipid classes. Most of their concentrations were consistent to the reference values in the previous studies.

CONCLUSION

The two methods for the transesterification of fatty acids from the seed of *Jatropha curcas* followed by Gas chromatography mass spectroscopy analysis can enable quick identification of various fatty acids in the seed samples of *Jatropha curcas*. Both the methods attempted are single step, without extraction of oil from seed. These FAMEs preparation methods resulted in a better recovery of the total fatty acid methyl esters present in *Jatropha* seed sample. These extraction procedures were found efficient, time saving as well as economical for the derivatization process involved for studying the fatty acids composition in the seeds of *Jatropha curcas*. These methods can be tested and applied to other oil seed crops as well as for other agricultural crops where handling of large number of samples are required for crop improvement and variety development programs. These methods may

not only be applied for oil seeds but can also be tested and used for any source which can be converted to methyl esters where oil extraction will become an additional step. These methods can facilitate large scale trials and make possible population studies. To develop biodiesel into an economically important alternative more research will be required for modifying the process to enhance the yield of ester.

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