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Chemometric analysis of Nicotiana tabacum FAME's using GC/MS, FT IR and NMR spectroscopic studies

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Abstract: Nicotiana tabacum commonly known as Tobacco plant which has broad application over soap, paint and cigar industries was now investigated for biodiesel production. Nicotiana tabacum seeds were one of the prominent sources for non-edible oil. Oil from Tobacco seeds were expelled with Soxhlet extraction apparatus using *n*-hexane as extraction solvent. Two stage trans-esterification has been adopted as the acid value of tobacco seed was higher. Standardization and characterization of the Tobacco seed biodiesel was accomplished by GC-MS, FTIR and NMR spectroscopy studies. Gas Chromatography and Mass Spectroscopy results indicated the existence of different fatty acids in the biodiesel and major constituent found among the fatty acids was linoleic acid. Stretching and bending signals of FT-IR spectrum revealed the methyl ester group presence in biodiesel. ^{1}H NMR and ^{13}C NMR analysis was also conducted for identification of different constituents and yield of biodiesel.
Keywords: GC-MS, FT-IR, Trans-esterification, Biodiesel, Soxhlet apparatus.

Introduction

Rising demand for conventional petro diesel, enormous increase of road vehicles along withemerging hazards of air pollutions has become a major threat which has ended up in finding an alternative fuel source. Recent studies explored that biodiesels derived from renewable sources of energy will be a promising alternative for conventional fuels, because of their unique features such as reduced emissions, self-lubricating property, lacking sulphur content and its availability from various renewable resources. Biodiesels derived from vegetable oils and animal fats have the similar properties of conventional fuels which states that biodiesel can be used on engines without any engine modification. Trans-esterification is used to convert vegetable oils into biodiesel. Depending upon the acid value of the oil single or two stage trans-esterification is preferred. Glycerol which is a by-product of trans-esterification also finds its application on soap and paint industries. As edible oils were already serving for food purpose, utilization of non-edible oils for biodiesel is more feasible. Biodiesels are non-toxic and biodegradable which can eliminate carbon monoxide (CO), Hydrocarbon (HC), Particulate matter and Sulphur dioxide (SO_x) upto a certain limit in emissions [2-7].

Gerhard Knothe investigated the stability of biodiesel upon the exposure to air due to the presence of unsaturated fatty acids in the biodiesel. GC-MS and ^{1}H NMR studies were conducted by employing commercial methyl soyate as biodiesel sample. Biodiesel surface area which falls upon the air has been kept as variable and tests were conducted by varying the surface area of the container. Fatty acid profile was determined by GC-MS technique, yet the ^{1}H NMR analysis also conducted to compare the data to other method results. As a result, it has been proved that biodiesel exposing to air tends to more oxidize more than the one which has less surface contact with air. Edible fats and Oils are usually determined by the existence of fatty acids and triglycerides [5].

Rohman et al. determined the properties and characteristics of lard mixed palm by conducting Fourier Transform Infrared Spectroscopy analysis. Lard mixed palm oil was utilized as sample for the FT-IR analysis. It showed the existence of triglycerides between the frequency regions of 1480cm^{-1} to 1085cm^{-1}[15]. Rania et al. examined the properties of Olive oil which was extracted using n-hexane as the extraction solvent in the Soxhlet apparatus. Sample oil dissolved in dimethyl sulphoxide solvent was used in^{1}H NMR determination. Recorded pattern of the ^{1}H NMR analysis defined the possible chemical shifts for different attributes of the oil sample [14].

Amerjothy et al. investigated various chemical components of Epaltesdivaricata (L.) Cass plant extracts using GC-MS analysis technique. Plant extracts obtained with the help of soxhlet apparatus using n-hexane, ethyl acetate, chloroform and alcohol separately as extraction solvent. In GC-MS a plot of intensity against retention time was obtained and the compounds are identified by comparing the data with NIST08 and WILEY08 library. Nine different chemical compounds were found between the retention times of 9.033 min to 32.300 min [1]. In order to identify the possible bioactive compounds from Wattakkavolubilis leaves, Usharani et al. extracted lipids on its leaves using soxhlet apparatus for 72hours at 50-60^{o}C. Final residue was examined using GC-MS analysis which indicated the existence of about 20 bioactive compounds including Dodeconoic acid, 9-Octadeconoic acid and Hexadeconoic acid as major compounds [20].

In this present investigation, biodiesel prepared from tobacco seed oil was characterized and standardized with GC-MS, FT-IR and NMR analysis. Tobacco seed oil was extracted from tobacco seeds using Soxhlet apparatus with n-hexane as the extraction solvent. Because of high acid content in tobacco seed oil, two stage trans-esterification was carried out to convert vegetable oil into biodiesel. Characterization and standardization of the Tobacco seed biodiesel was conducted with GC-MS, FT-IR and NMR spectroscopic studies.

Materials and Methods

5kg of Hybrid Tobacco seeds (Abirami variety) was procured from Central Tobacco Research Institute (CTRI), Vedasandur, Dindugal District, Tamil Nadu, India. The seeds were dried in sunlight for 48hrs to remove moisture content and crushed in a grinding mill. Grounded seeds are then placed on a whatman thimble made up of fine cellulose membranes in the extraction unit of soxhlet apparatus. Optimum temperature of 50^{o}-60^{o}C was maintained in the reaction chamber as the boiling point of n-hexane was noticed to be 64.7^{o}C. Cyclic extraction process was allowed to occurs upto 120minutes followed by vacuum distillation of n-hexane with rotary evaporator with a recovery efficiency of 90%.

Gas Chromatography and Mass Spectroscopy (GC-MS) analysis was conducted on JOEL GCMATE II system which has an enlarged resolution accompanied with dual focus capability. System contains maximum resolution of 6000 and 1500 Daltons of maximum calibrated mass. Thesystem also comprises the possibility to study molar mass determination, structural analysis, structural elucidation of organic compounds and fragmentation process. Data acquisition system to plot the graph and auto sampler adds features to the spectrometry study.Fourier Transform Infrared Spectroscopy (FT-IR) test was carried out with PERKIN ELMER SYSTEM ONE FT-IR/ATR system enhanced with globar and mercury vapour lamp as sources and an interferometer chamber comprising of beam splitter. Vibrational motion exhibited by the molecules has been made use for characterization of organic, inorganic and biological compounds. Perkin Elmer spectrum also comprises of a wider scan range between 450cm^{-1} to 4000cm^{-1}is used for quantitative estimation of certain compounds.

BRUKER ADVANCE III 500MHz NMR multi nuclei solution spectrometer empowered with super conducting 11.7 Tesla magnets, passively shielded by a base extended from 5 to 5.4cms along with inbuilt cryo-shims and 34 channel room temperature shims. RF console compatible field gradients with low heat dissipation exists to carry out the triple resonance experiments, phase resolution better than 0.1degree, frequency resolution better than 0.1Hz and observation of nuclei including 19F. Amplified power range of maximum 300W and a minimum 100W is supplied. TOPSPIN–2 software for processing the data with auto sampler capable upto 24 samples was used [11,13].

Results and Discussion

Gas Chromatography-Mass Spectroscopy

Gas Chromatography and Mass Spectroscopy (GC-MS) analysis disclosed the existence of saturated and unsaturated fatty acids in the Tobacco seed biodiesel. Mass spectra plots are compared with National Institute of Standards and Technology (NIST-MS I) library in order to determine the fatty acids and identified components were tabulated with respect to their retention time, scans and number of ions in Table (1). On GC-MS profile shown in the Figure (1), 9 stretching peaks at different retention time indicates the fatty acids likeTridecyclic acid, Pentadecyclic acid, Linoleic acid, Margaric acid, Linolelaidic acid, Eicosanoic acid, Gondoic acid, Arachidic acid and Behenic acid presence in the biodiesel. At retention time 18.85, a peak has extended much which defines the linoleic acid as a major constituent of the tobacco seed biodiesel and margaric acid was next major constituent of the tobacco seed biodiesel which was extended till 17.17 min retention time [12,16-18,21].

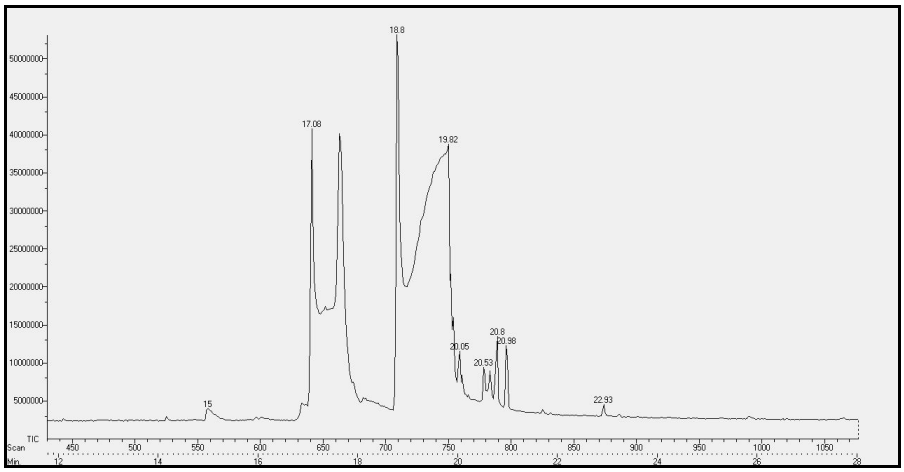

Figure 1. GC/MS spectrum of Nicotiana tabacum biodiesel

Table 1. Fatty Acid Methyl Esters composition of Nicotiana tabacum biodiesel

Retention time (RT)	Name of the ester	Name of the fatty acid	Number of Ions	Scan	Molar Mass (g/mol)
15.00	Tridecanoic acid, 12-methyl-, methyl ester	Tridecyclic acid	1666	558	122.12
17.17	Pentadecanoic acid, 14-methyl-, methyl ester	Pentadecyclic acid	1255	644	242.39
18.85	9,12-Octadecadienoic acid [Z,Z]-, methyl ester	Linoleic acid	1050	711	28.445
19.85	Heptadecanoic acid, 15-methyl-, methyl ester	Margaric acid	1245	751	270.45
20.05	9,12-Octadecadienoic acid, ethyl ester	Linolelaidic acid	1348	759	280.45
20.53	6,11-Eicosadienoic acid, methyl ester	Eicosenoic acid	1430	778	310.51
20.80	11-Eicosenoic acid, methyl ester	Gondoic acid	1281	789	310.51
20.98	Eicosanoic acid, methyl ester	Arachidic acid	1371	796	312.53
22.93	Docosanoic acid, methyl ester	Behenic acid	1540	874	340.58

Fourier Transform Infra-Red Spectroscopy

Figure (2) indicates the FT-IR spectroscopy analysis of tobacco seed biodiesel scanned with the frequency range between 4000cm^{-1} and 450cm^{-1}. Fourier Transform Infrared Spectroscopy of Tobacco seed biodiesel defines the existence of different functional group in induced sample.

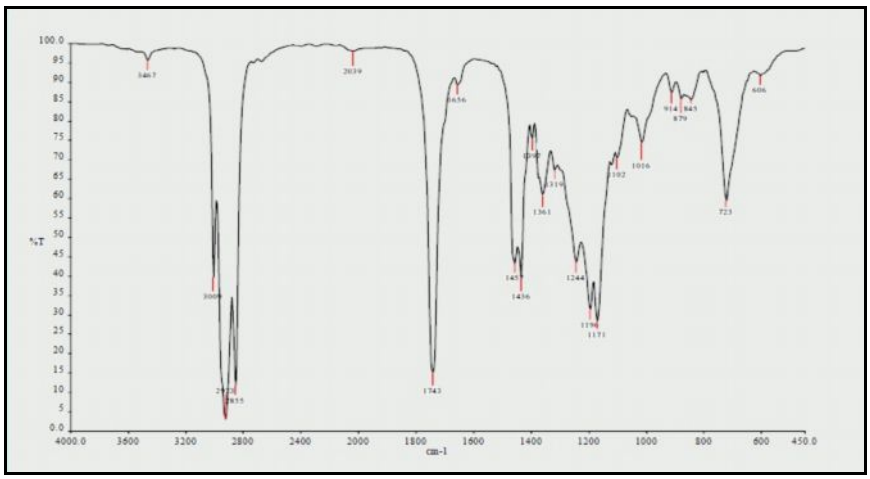

Figure 2. FT IR spectrum of Nicotiana tabacum biodiesel

A peak with bending and stretching between 723cm^{-1} and 879cm^{-1} shows the existence of alkene group [C=C]. Strong stretching between the 1016cm^{-1} and 1196cm^{-1} proves the existence of methyl ester group on tobacco seed biodiesel. Within the interval of frequency range from 1319cm^{-1} and 1457cm^{-1}, existence of carboxylic acid was identified on comparison with the library. A medium stretch extending from 1656cm^{-1} to 1743cm^{-1} shows the unsaturated ester [C=O]. Presence of alkane was also confirmed with a medium peak stretching between the frequency range 2855cm^{-1} to 3009cm^{-1}[8,10,19].

^{1}H NMR (Nuclear Magnetic Resonance)

Tobacco seed biodiesel was characterised by Nuclear Magnetic Resonance analysis. Methanol D4 (CD_3OD) was used as solvent which has a melting point of -97.8^{o}C and boiling point of 64.6^{o}C. Chemical shift assignment for tobacco seed biodiesel was shown in Figure (3).

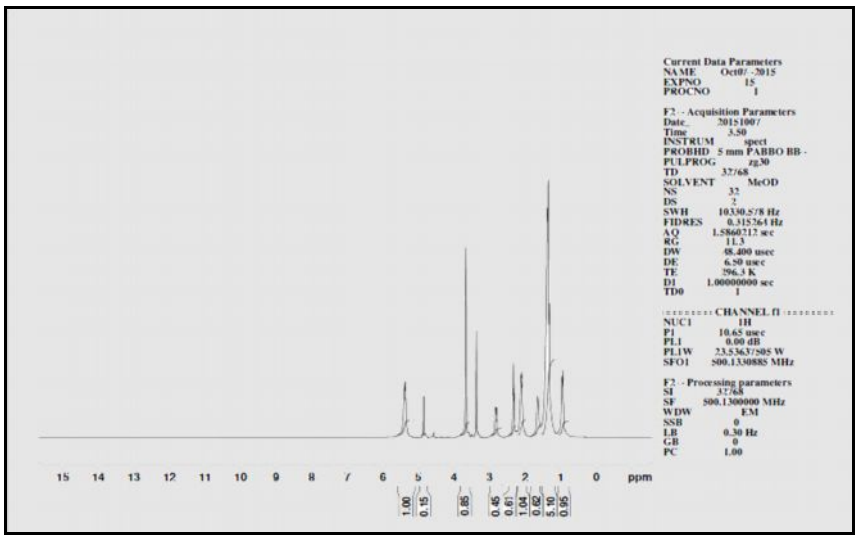

Figure 3. ^{1}H spectrum of Nicotiana tabacum biodiesel

The intensity of the chemical shift and its resonance indicates the information of chemical combinations in the sample induced. Obtained chemical shifts are defined in ppm. A peak signal obtained at 1.35ppm and

2.32ppm existing as triplet defines the presence of methyl ester group in biodiesel. Another peak at 0.94ppm indicates the methyl group of linoleic acyl chain. No projections were found beyond the 5.5ppm which may be because of lack of some biodiesel constituents [9].

^{13}C NMR (Nuclear Magnetic Resonance)

Characteristics of Tobacco seed biodiesel expressed with ^{13}C NMR analysis was shown in Figure (4). Tobacco seed biodiesel interpreted with chemical shifts of ^{13}C NMR was specified between the ranges of 0 to 200ppm. Weak signal at 13.31ppm, 13.68ppm and 25.31ppm represents the presence of methyl group of methyl esters. A strong signal at 47.78ppm and 17.39ppm indicates the carbonyl existence which is the unsaturation in the methyl esters. Some peaks at 54ppm are may be due to the presence carbon in the methyl groups.

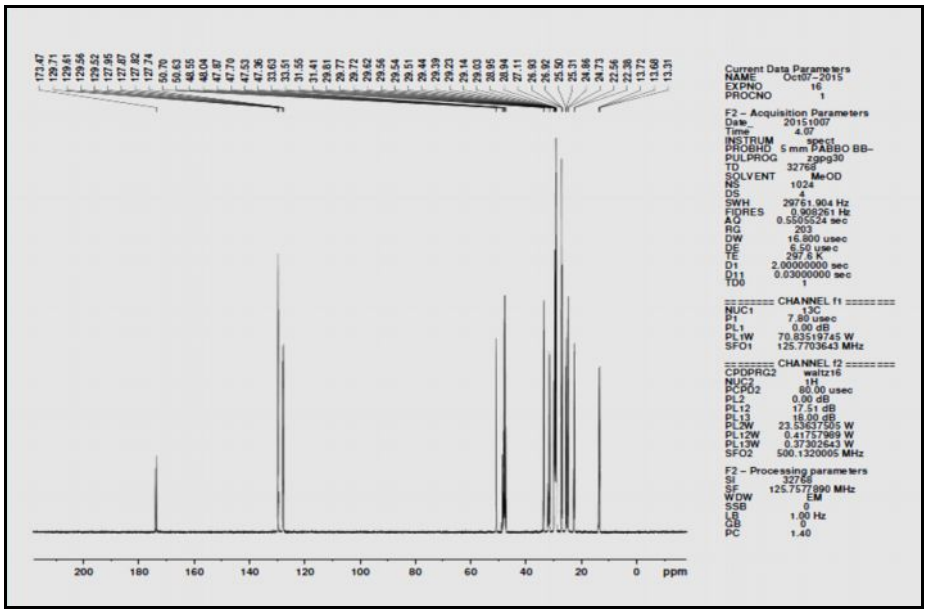

Figure 4. ^{13}CSpectrum of Nicotiana tabacum biodiesel

Conclusion

In this present study of characteristics of the Tobacco seed biodiesel with spectroscopic investigation the following observations were concluded,

- Extraction of the Tobacco seed oil using soxhlet apparatus with n-hexane as an extraction solvent.
- Two stage trans-esterification was carried out with KOH as catalyst and 1:6 molar mass ratio for methanol and oil
- GC-MS spectrum indicates the existence of nine differentfatty acids in the tobacco seed biodiesel.
- Fourier Transform Infrared Spectrometry results has confirmed the presence of fatty acid methyl esters with the bending and stretching peaks scanned between the frequency range of 4000cm^{-1} to 450cm^{-1}.
- ^{1}H NMR visualized the fatty acid methyl esters by showing a signal at 1.35ppm and 2.32ppm. ^{13}C NMR showed the chemical shifts which defines the existence of the methyl and carbonyl group on biodiesel.

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