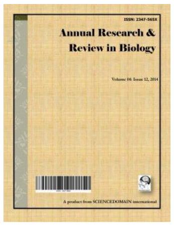

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Bio-diesel Production from Residual Sunflower Oil by Trans-esterification (Acidic, Alkaline Enzymatic) and Analysis of Some of Its Physico-chemical Parameters

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Authors' contributions

This work was carried out in collaboration between both authors. Author STG designed the study and wrote the protocol. Author DK performed the study. Authors STG and DK managed the analyses of the study, wrote the first draft of the manuscript and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Recycling of residual sunflower oil for bio-diesel production, determination, and comparison with some of the physico-chemical parameters with the jatropha bio-diesel and ASTM D6751and EN 14214 standards.
Study Design: Experimental observation with production of bio-diesel and characterization of some analytical parameters.
Place and Duration of the Study: Department of Microbiology and Biotechnology, Jnana Barathi Campus, Bangalore University, Bengaluru 560056, July 2015 to June 2016, India.

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Methodology: Acidic, Alkaline and Enzymatic trans-esterification was carried out with the residual sunflower oil for bio-diesel production by using the catalysts: HCl, NaOH and immobilized lipase enzyme (Hi-media RM1265) respectively. Some physico-chemical parameters of bio-diesel obtained in these processes were compared with the jatropha bio-diesel and ASTM, EN standards. The efficiency of the bio-diesel of the residual sunflower oil was analyzed by Fourier transform infrared spectroscopy technique (FTIR).

Results: The bio-diesel produced from residual sunflower oil by three different processes was analyzed for physico-chemical parameters: Density, Viscosity, Acid value, Cetane number, Iodine value and Flash point. By comparing these parameters with referral jatropha bio-diesel and ASTM D6751, PS121, EN 14214. The Cetane number (CN) responsible for auto-ignition of the sunflower bio-diesel was found to be more than 50 CN when compared with the jatropha bio-diesel (CN=47-50). The efficiency of the bio-diesel was assessed by using Fourier transform infrared spectroscopy technique. This showed that alkaline method was more effective when compared to the acidic and enzymatic methods in terms of more fatty acid ester formation.

Conclusion: The study parameters of produced bio-diesel from residual sunflower oil evinced suitability for recycling purpose. Alkaline trans-esterification method for bio-diesel production was cost effective and efficient as per Fatty Acid Methylesters content (FAMEs). Hence, it lays a foundation for the further research to utilize residual sunflower oil as an alternative fuel.

Keywords: Sunflower; bio-diesel; jatropha; cetane number; lipase; flash point.

1. INTRODUCTION

The coal and petroleum are non-renewable fossil fuels of nature and are used abundantly all over the world. Hence, are accepted as unsustainable due to the depleting resources. The extensive use of fuels in automobiles has led to the accumulation of carbon dioxide – greenhouse gases. The concentration of the carbon dioxide has exceeded the safer level of 350 ppm and reached dangerous levels of 450 ppm, which contributes significantly to the global warming. To overcome these problems, in recent years bio-fuel has drawn an attention as a sustainable energy source that may cope up as an alternative fuel [1-2].

Bio-fuels are the major alternative sources of energy accounting for more than 90% of the energy consumption in poor developing countries [2]. Several public institutions, state bio-fuel boards, state agricultural universities and cooperative sectors are also supporting the bio-fuel mission in different capacities. Bio-fuels include bio-ethanol, bio-methanol, bio-diesel, bio-gas, biosynthetic gas (bio-syngas), bio oil, bio-char and bio-hydrogen. The traditional way of production of bio-fuels was ethanol from corn, wheat, sugar beets or of cane and bio-diesel from vegetable oils. The fatty acid content of edible and non-edible oils is suitable for the production of bio-diesel as they minimize the release of carbon monoxide, nitrogen oxide, and sulfur to the environment hence gaining popularity. The utilization of waste vegetable oils for bio-diesel production by trans-esterification process is one of the great environmental concern as they reduce the health hazards in human beings [3].

Several investigations are intended to re-consideration of non-edible oils and used vegetable oil from catering industries for bio-diesel production. The various properties associated with them are measured to find out the utility by comparing with reference of conventional jatropha bio-diesel [4].

Bio-diesel from sunflower oil has minimal impact on the domestic use of sunflower oil provided that the crop is grown commercially for use in both applications. There are few research reports available about the usage of sunflower oil as an alternate option as a blend with petro-diesel in suitable proportions [5]. Bio-diesel production from sunflower oil using calcium oxide [6-7], sunflower oil subjected to the trans-esterification with calcium oxide in supercritical methanol for obtaining bio-diesel [8] and also sunflower oil subjected for bio-diesel production using industrially important lipase enzyme from *Candida antarctica* are known [9-10].

Similarly, few reports are also available on the usage of used sunflower oil for bio-diesel production where they reported on the acid number, viscosity and phosphorus content [11] and production by using immobilized lipase enzyme as catalyst [12].

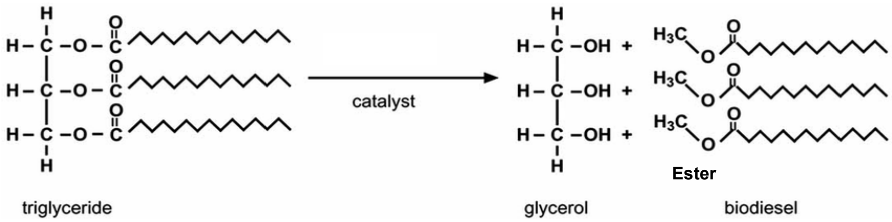

Fig. 1. General Trans-esterification reaction

Fatty acid contents in the oil also plays a major role in feedstock selection, as per the literature, the fatty acid content in jatropha oil are Oleic acid 44.7%, Linolenic acid 32.8%, Palmitic oil 14.2% and steric acid 7% similarly sunflower oil contains Linoleic acid 66.2%, Oleic acid 21.1%, Palmitic acid 5.8% and steric acid 4.5% [13]. Hence by considering the above facts, the present study was designed with an aim of reuse of residual sunflower oil by purification and trans-esterification to produce methyl or ethyl esters by acidic, alkali and enzymatic methods as shown in Fig. 1. And also by comparing the physico-chemical parameters such as viscosity which is responsible for injector lubrication, acid value for the presence of free fatty acids, Cetane number for combustion efficiency, iodine value for the degree of unsaturation and Flash point in influencing the fuel efficiency with jatropha bio-diesel and ASTM D6751-PS121, EN 14214 standards [14-15]. And determining the efficiency of the obtained bio-diesel ascertained by Fatty acid methyl ester content by FTIR analysis. The characterization of physic chemical parameters of residual sunflower oil helpful to evaluate the raw material source and its quality on the amount of methylesters yield.

2. MATERIALS AND METHODS

2.1 Reagents and Standards

Immobilized Lipase enzyme RM1265 obtained from Hi-media laboratories. All other chemicals like hydrochloric acid, anhydrous sodium hydroxide, n-hexane, and methanol are of Analytical grade obtained from S.D fine chemicals limited and Nice chemicals private limited. Jatropha bio-diesel used as referral bio-diesel was collected from Prof. Balakrishne Gowda's lab, Gandhi Krishi Vijnana Kendra Bangalore.

2.2 Collection and Purification of the Residual Oil

The used sunflower oil procured from the restaurants contained some impurities. This residual oil was subjected to filtration through glass wool packed in the separating funnel, followed by filtration using Whatman paper for removal of impurities. The impurity free oil was collected in a beaker and used for trans-esterification.

2.3 Acidic Trans-esterification

Trans-esterification of residual sunflower oil was carried out in 150 mL Erlenmeyer flask. A volume of 10 mL of the sample was heated at 100ºC in a water bath for 15 minutes followed by the addition of 15 mL methanol and 3 mL of HCl added as acidic catalyst. To this reaction mixture, 10 mL of n-hexane was added with vigorous shaking and incubated for 24 hours at room temperature. The reaction mixture was subjected for separation using separating funnel. Thus obtained top most layer containing methylesters as bio-diesel and the bottom layer was glycerol were separated. The collected bio-diesel layer was subjected to Fourier Transform Infrared (FTIR) spectroscopic analysis and determined the physico-chemical properties by comparing it with ASTM and EN standards [16].

2.4 Alkaline Trans-esterification

Trans-esterification of residual sunflower oil was carried out in 150 mL Erlenmeyer flask. A volume of 10 mL of the sample was heated at 55°C in water bath for 15 minutes followed by the addition of sodium methoxide i.e. 1.4 g of NaOH in 80 mL of methanol which act as a basic catalyst. To this reaction mixture, 10 mL of n-hexane was added with vigorous shaking and incubated for 24 hours at room temperature. The reaction mixture was subjected for separation using separating funnel. Thus obtained top most layer containing methylesters as bio-diesel and the bottom layer glycerol were separated. The collected bio-diesel was subjected to FTIR analysis and determined the physico-chemical properties by comparing it with ASTM and EN standards [17].

2.5 Enzymatic Trans-esterification

The enzymatic trans-esterification of the residual oil was carried out on incubator shaker at 200 rpm for a period of 48 hours at 40ºC. 10 mL of the residual oil was taken in Erlenmeyer flask, 13.33 mL of ethanol fraction (1:4 of oil to ethanol) was added every one third of the reaction time. Once the first fraction of ethanol was added 400 mg of the immobilized lipase was added. After the addition of the last fraction of the ethanol, 0.6 mL of n-hexane was added and allowed for the reaction to take place in an incubator shaker, the reaction mixture was separated using separating funnel. Top most layer containing methylesters as bio-diesel and bottom layer as glycerol was separated. The obtained bio-diesel was subjected for FTIR analysis and physico-chemical parameters were determined by comparing it with referral jatropha, ASTM and EN standards [18].

2.6 Analytical Characterization of Bio-diesel Produced

The saponification process was carried out to determine the presence of free and combined fatty acids present in the oil [19]. The other physico-chemical parameters include measurement of Density by pycnometer and viscosity by viscometer at 24°C which is used to measure the internal fluid friction responsible for injector lubrication. Determination of saponification value for the measure the presence of combined and free fatty acid content at 24°C by titrimetric method [20]. Estimation of acid value gives the measure of number of carboxylic groups in a chemical compound which was measured by titrimetric method against known concentration of potassium hydroxide (KOH) using phenolphthalein as an indicator. Acid value should be lower than 0.50 mg KOH/g since the FFA produced might corrode the automotive parts which cause damage to vehicle engines and fuel tanks [21]. Iodine value is often used to determine the amount of unsaturation in fatty acids by Hannus reaction using titrimetric method [22-23]. Higher iodine value represents more C=C bonds. And cetane number (Hexadecane number) represents inverse function of fuel ignition delay. It indicates the ignition properties of the diesel. Higher cetane number indicates that fuel have shorter ignition delay periods, than the fuels having lower cetane number. Flash point is the minimum temperature of bio-diesel at which it ignites and determines the fuel efficiency measured by Pensky-martens flash point apparatus. Cetane number is the readiness of the fuel to auto-ignite when injected into engine, measured by Near infra-red analyzer method adopted by analytical laboratory Bengaluru.

Ester content: FTIR is a technique used to obtain an infrared spectrum of absorption or emission of a bio-diesel spectral resolution data over a wide spectral range analyzed for Fatty acid methyl ester. FTIR analysis was done in department of organic chemistry central college using BRUKER FTIR (model name: IFS 125HR).

3. RESULTS AND DISCUSSION

3.1 Comparative Results on Physico-Chemical Properties of Bio-Diesel with the Standards

The free and combined fatty acid content of the residual sunflower oil was determined for saponification value and found to be 192 mg KOH/g in residual sunflower oil and 197 mg KOH/g in jatropha oil. The bio-diesel obtained from residual sunflower oil had its efficiency checked to be used as an alternative bio-fuel after trans-esterification. The physico-chemical parameters were determined and compared with referral Jatropha bio-diesel and ASTM D6751-PS121 and EN standards [24].

In order to be used as bio-diesel, the physico-chemical parameters (Table 1) of the obtained trans-esterified bio-diesel must comply with a series of ASTM and EN standards. The viscosity responsible for the operation of the fuel injection equipment and injector lubrication [25-26], Iodine value for evaluating degree of unsaturation that determine the stability to oxidation in the bio-diesel [27-28], acid value represents free fatty acids was also determined to check the acid moieties in the bio-diesel [29], were measured. Cetane number for combustion efficiency was analyzed to check for the delay period in the bio-diesel, flash point was determined to know the fuel efficiency in terms of flammable vapors which can be ignited in air by a flame above its surface. By the comparative study of the above methods parameters, the unique observation was found to be the Cetane number showing more than 50 CN whereas standard jatropha showed 40-50. The bio-diesel obtained from alkaline method was found to have higher efficiency when compared with the referral jatropha bio-diesel and ASTM and EN standards thus, can be used as alternative source of bio-diesel. Also the alkaline base used in the trans-esterification acts as catalyst but not reactants hence alkaline used is not consumed by trans-esterification reaction. And also the reaction did not take place at elevated temperature minimizes the soap formation, therefore alkaline trans-esterification is sustainable and stays the best method for bio-diesel production. Whereas bio-diesel obtained from enzymatic method was found to have good efficiency when compared, but requires longer duration and is exorbitant to be used as alternative fuel. Whereas, the bio-diesel obtained from acidic method showed variation in some physico-chemical parameters and was found to be not efficient when compared with the standards (Figs. 2-4).

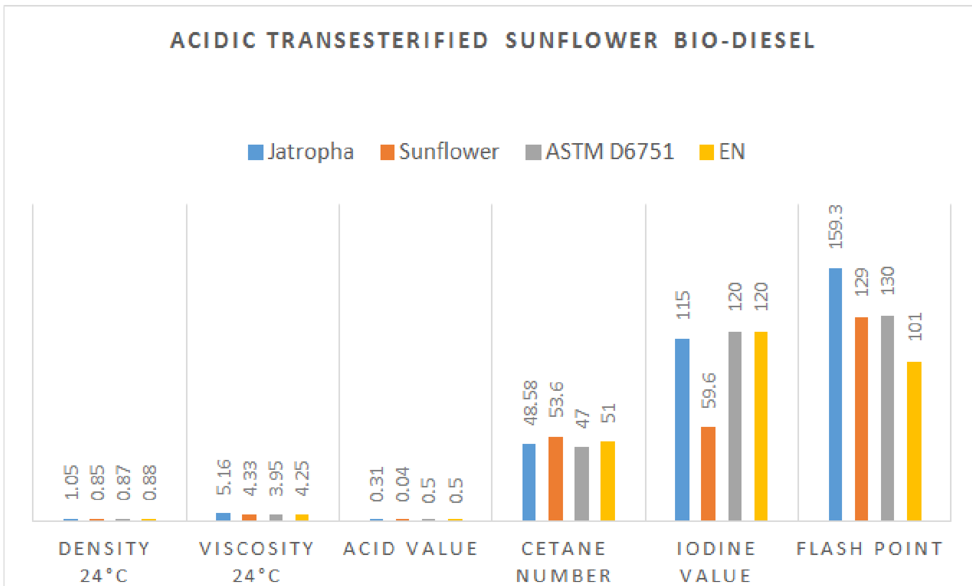

Fig. 2. Physico-chemical properties of sunflower bio-diesel produced by acidic trans-esterification, in comparison with jatropha bio-diesel, EN and ASTM standards

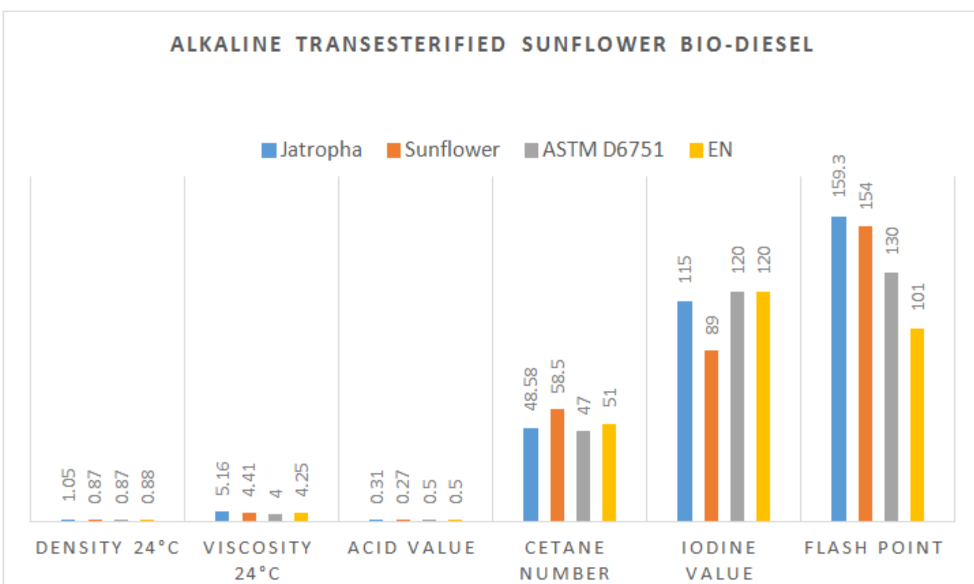

Fig. 3. Physico-chemical properties of sunflower bio-diesel produced by alkaline trans-esterification, in comparison with jatropha bio-diesel, EN and ASTM standards

Table 1. Physico-chemical properties of the sunflower residual oil bio-diesel, with comparative standards

				Bio-diesel produced by trans-esterification		
Parameters	**Comparative standards (ASTM D6751*/ ASTM PS121 **)**	**European Standards 14214**	**Jatropha standards**	**Acidic method**	**Alkaline method**	**Enzymatic method**
				Sunflower	**Sunflower**	**Sunflower**
Density 24°C (g/cc)	0.87**	0.86-0.90	1.05	0.85	0.87	0.94
Viscosity 24°C (mm^2/sec)	1.9-6*	3.5-5.0	5.16	4.33	4.41	5.66
Acid value (mg KOH1/g)	Max 0.50*	0.5	0.31	0.04	0.27	0.33
Cetane number	Min 47*	51	48.58	53.6	58.5	58.3
Iodine value (g/100 g)	Max 120*	120	115	59.6	89	138
Flash point (ºC)	Min 130*	101	159.3	129	154	130

3.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis (Figs. 5-8) was carried out to check the presence of FAMEs which determine the efficiency of the bio-diesel to be used as alternative source of fuel.

The FTIR analysis revels the characteristic peaks on a bio-diesel spectrum are one at 1000-1300 cm^{-1} which is related to O-CH3 vibrations. Comparing Figs. 5-8 shows that the peak characterized by O-CH3 vibrations is prominent in all spectra. The peak gives an indication of the attachment of the alkali group of the alcohol to

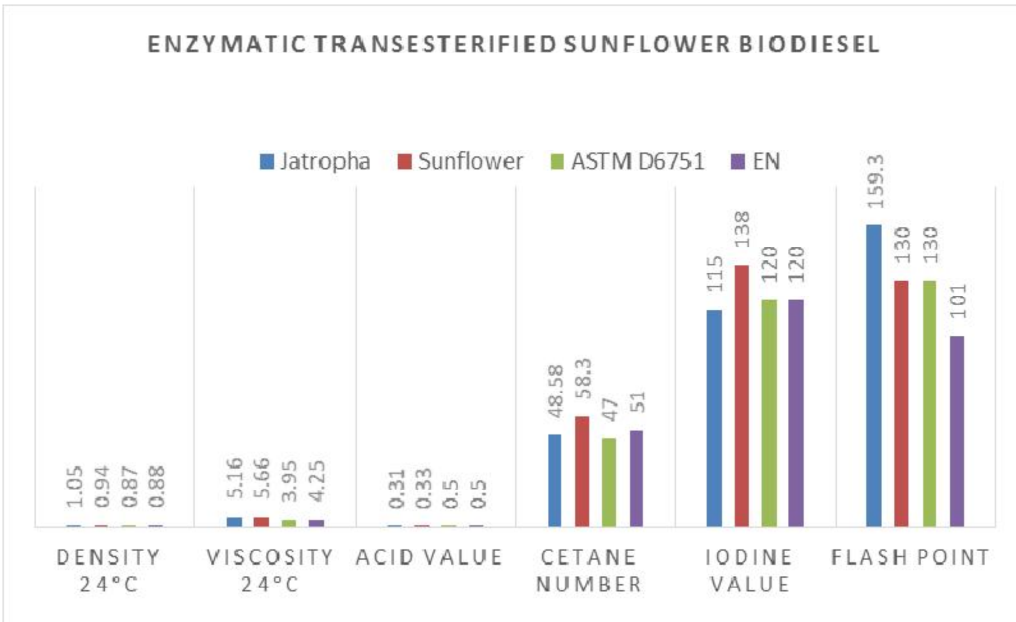

Fig. 4. Physico-chemical properties of sunflower bio-diesel produced by enzymatic trans-esterification, in comparison with jatropha bio-diesel, EN and ASTM standards

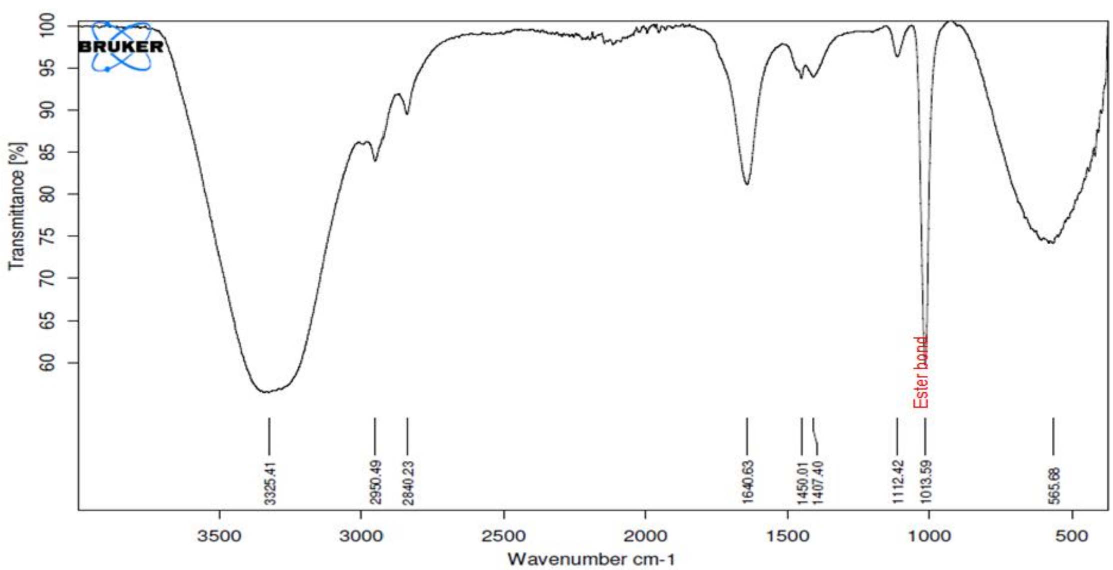

Fig. 5. FTIR spectrum of sunflower bio-diesel, obtained by acidic trans-esterification process for FAME's analysis

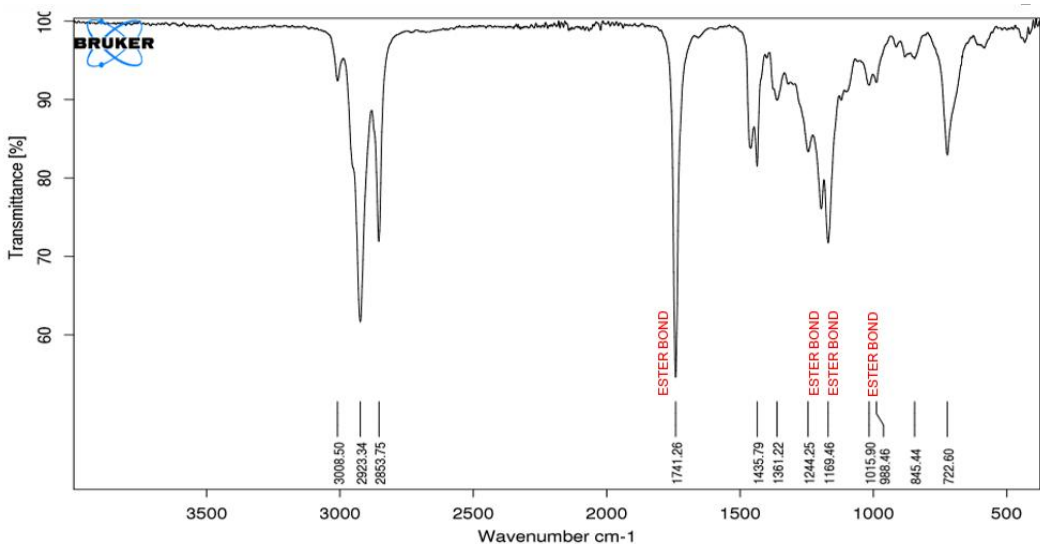

Fig. 6. FTIR spectrum of sunflower bio-diesel, obtained by from alkaline trans-esterification process for FAME's analysis

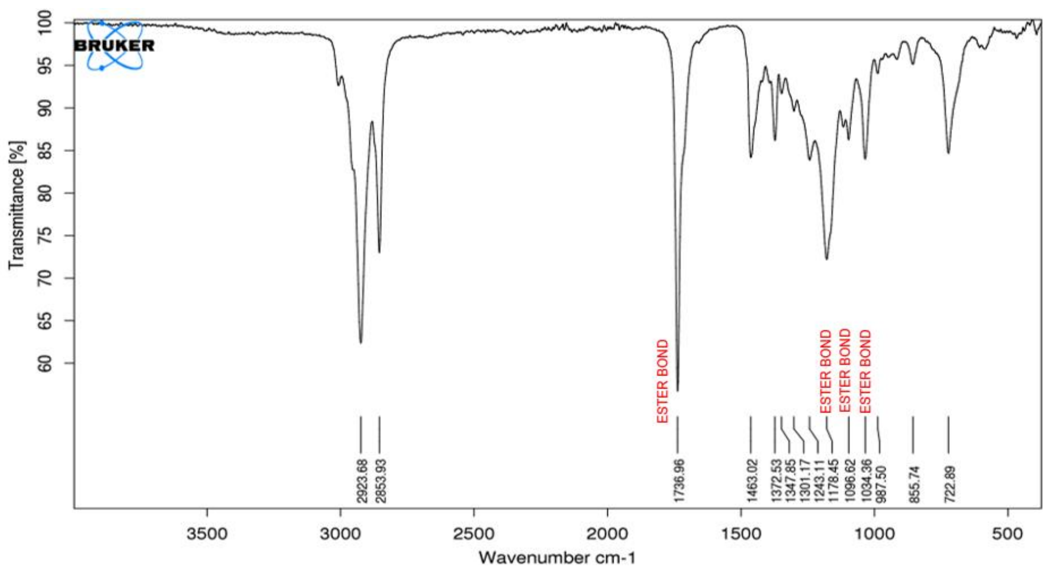

Fig. 7. FTIR spectrum of sunflower bio-diesel, obtained by from enzymatic trans-esterification process for FAME's analysis

the fatty acid group in the triglycerides, those forming esters. In addition there are bonds appearing between 1150-1450 cm^{-1} attribute to C-O ester stretching vibrations and 1735-1750 cm^{-1} both attribute to C=O ester stretching vibrations. 1000-1400 cm^{-1} attribute to alkyl halide C-F stretching vibrations, 1080-1360 cm^{-1} attribute to amine group C-N stretching vibrations, 2850-3000 cm^{-1} attribute to alkanes C-H stretching vibrations, 1720-1740 cm^{-1} attribute to aldehyde group C=O stretching vibrations, 1670-1820 cm^{-1} attributes to ester carbonyl C=O stretching vibrations, 2500-3300 cm^{-1} attributes to acid group O-H stretching vibrations and 1400-1600 cm^{-1} attributes to aromatic groups C=C stretching vibrations. The analysis confirms the purity of the respective bio-diesel samples with the presence of methyl ester groups. Thus presence of FAMEs plays an important role in determining the bio-diesel efficiency.

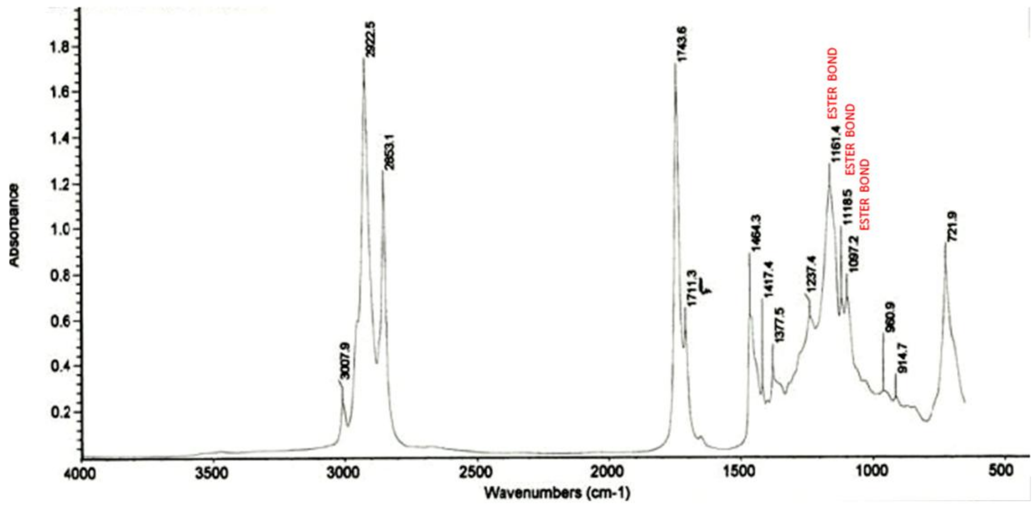

Fig. 8. FTIR spectrum of jatropha bio-diesel, obtained by from enzymatic trans-esterification process for FAME's analysis (used as comparative standard)

From the above obtained FTIR peaks, sunflower bio-diesel from alkaline and enzymatic method were found to be effective with the presence of good amount of methylesters when compared with the acidic method. Whereas, alkaline method is cost effective and easy to handle process and enzymatic method is time consuming. Thus alkaline method holds the suitable method.

The fatty acid methylesters are the key parameter to denote the bio-fuel efficiency [30]. Based on the FAMEs analysis (Figs. 5-8) the bio-diesel obtained from alkaline method and enzymatic method had good amount of fatty acid esters and was found to be similar with standard jatropha bio-diesel. Bio-diesel obtained by these methods has the potential to be used as an alternative source of fuel in the form of blends. Alkaline method is cost effective and easily produced whereas the enzymatic method is costly and requires longer duration for bio-diesel production. However acid method was found to contain the least amount of FAMEs and is not efficient to be used as bio-fuel as it does not meet the standard criteria's.

4. CONCLUSION

The bio-diesel obtained by alkaline trans-esterification of residual sunflower oil by virtue of more fatty acid methyl ester content and other physico-chemical properties showed as better alternative with good fuel efficiency in terms of cetane number. This bio-diesel has the capability to act as an alternative fuel to jatropha bio-diesel used as blends. The production of bio-diesel from residual sunflower oil minimizes the environmental oil dumps that cause soil and water pollution. Further, it lays a foundation for the further research to be carried on for optimal use as an alternative fuel.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Peer M. Schenk, Skye R, Thomas-Hall, Evan Stephens, Ute C. Marx, Jan H. Mussgnug, et al. Second generation bio-fuels: High-efficiency microalgae for bio-diesel production. Bioenergy Res. 2008;1: 20–43.
   DOI: 10.1007/s12155-008-9008-8
2. Anonymous. Bioenergy. Sustainable Development Department, FAO, Rome, Italy. FAO; 2005.
   (Accessed on April 11, 2006)
   Available:http://www.fao.org/sd/dim_en2/en2_050402_en.htm
3. George Anastopoulos, Ypatia Zannikou, Stamoulis Stournas, Stamatis Kalligeros. Trans-esterification of vegetable oils with ethanol and characterization of the key fuel properties of ethyl esters. Energies. 2009; 2:362-376.
   DOI: 10.3390/en20200362

4. Pugazhenthi R, Chandrasekaran M, Muthuraman RK, Vivek P, Parthiban A. Comparative characteristic study of *Jatropha* and cardanol bio-diesel blends. International Conference on Emerging Trends in Engineering Research IOP Publishing IOP Conf. Series. Materials Science and Engineering. 2017;183 012038:1-10.
DOI: 10.1088/1757-899X/183/1/012038
5. Thirumarimuruga M, M.shivashankar V, Merly A. Xavier, Prabhakaran D, Kannadasan T. Preparation of bio-diesel from sunflower oil by trans-esterification. International Journal of Bioscience Biochemistry and bioinformatics. 2012; 2(6):441-444.
DOI: 10.7763/IJBBB.2012.V2.151
6. Antolin G, Tinault FV, Bricerio Y, Castario V, Perez C and Ramierz AI. Optimization of bio-diesel production by sunflower oil. Bio Source technology. 2002;83(2):111-114.
DOI: 10.1016/S0960-8524(01)00200-0
7. Lopez Grandos M, Zafra Poves MD, Martin Alonso D, Mariscal R, Cabello Galisteo F, Moreno-Tost R, et al. Bio-diesel from sunflower oil by using activated calcium oxide. Applied catalysis B: Environmental. 2007;73(3):317-326.
DOI: 10.1016/j.apcatb.2006.12.017
8. Ayhan Demirbas. Bio-diesel from sunflower oil in supercritical methanol with calcium oxide. Energy Conversion and Management. 2007;48(3):937-941.
DOI: 10.1016/j.enconman.2006.08.004
9. Belazi-Bako K, koracs F, Gubicza L, Hanesok J. Enzymatic bio-diesel production from sunflower oil by candida antarctica lipase in a solvent free system. Biocatalysis and biotransformation. 2002; 20(6):437-439.
DOI: 10.1080/1024242021000040855
10. Raza S, Fransson L, Hult K. Enantioselectivity in *Candida antarctica* Lipase B: A molecular dynamics study. Protein Science. 2001;10(2):329-338:
DOI: 10.1110/ps.33901
11. Demirbas A. Bio-diesel production from vegetable oils via catalytic and non-catalytic supercritical methanol trans-esterification methods. Energy and Combustion Science. 2005;466-487:
DOI: 10.1016/j.pecs.2005.09.001
12. Dizge N, Aydiner C, Imer DY, Bayramoglu M, Tanriseven A, Keskinler B. Bio-diesel production from sunflower, soybean, and waste cooking oils by trans-esterification using lipase immobilized onto a novel microporous polymer. Bioresource Technology. 2009;100(6):1983-1991.
DOI: 10.1016/j.biortech.2008.10.008
13. Emil akbar, Zahira Yaakob, Siti Kartom Kamarudin, Manal Ismail, Jumat Salimon. Characteristic and composition of *Jatropha curcas* oil seed from Malaysia and its potential as bio-diesel feedstock. European Journal of Scientific Research. 2009; 29(3):396-403.
14. Boyce AN, Hossain ABMS. Bio-diesel production from waste sunflower cooking oil as an environmental recycling process and renewable energy. Bulgarian Journal of Agricultural Science. 2009;15(4):312-317.
15. Shine K, Tyson, Paul J. Nazzaro. Bio-diesel as a heating oil blend stock, Proceedings of the 2001 National Oilheat Research Alliance Technology Conference. BNL-52625; 2001.
Available:https://searchworks.stanford.edu/view/11338261
16. Kenichi Ichihara, Yumeto Fukubayashi. Preparation of fatty acid methylesters for gas-liquid chromatography. A Journal of Lipid Research. 2009;51:634-640:
DOI: 10.1194/jlr.D001065
17. John Bush. Trans-esterification lab procedure using alkali catalyst and methanol used for processing RBD vegetable oils (unused cooking oil/food grade vegetable oil) or other oils containing very low amounts of free fatty acids. 2005; 1-2.
Available:https://inside.mines.edu/~jobush/gk 12/lessons/Bio-diesel.pdf
18. Elizabeth Funmilayo Aransiola. Lipase catalysed ethanolysis of *Jatropha* oil for bio-diesel production. Energy and Environment Research. 2013;1(3):85-92:
DOI: 10.5539/eer.v3n1p85
19. Sivaramakrishnan K, Ravikumar P. Determination of cetane number of bio-diesel and its influence on physical properties. ARPN Journal of Engineering and Applied Sciences. 2012;7(2):205-211.
Available:http://www.arpnjournals.com/jeas/research_papers/rp_2012/jeas_0212_640.pdf
20. Mittelbach M. Diesel fuel derived from vegetable oils, IV: Specifications and quality control of bio-diesel. Bioresource Technology. 1996;56:7-11.
DOI: 10.1016/0960-8524(95)00172-7

21. Gerhard Knothe. Dependence of bio-diesel fuel properties on the structure of fatty acid alkyl esters. Fuel processing Technology. 2005;86(10):1059-1070. doi.org/10.1016/j.fuproc.2004.11.002
22. Gopinath A, Sukumar Puhan, Nagarajan G. Theoretical modeling of iodine value and saponification value of bio-diesel fuels from their fatty acid composition. Renewable Energy. 2009;34(7):1806-1811. DOI: 10.1016/j.renene.2008.11.023
23. Hiscox DJ. Determination of Iodine Numbers. Anal. Chem. 1948;20(7):679–680. DOI: 10.1021/ac60019a030
24. Van Gerpen F. Cetane number testing of bio-diesel. Liquid fuels and industrial products from renewable resources—Proceedings of the Third Liquid Fuels Conference, Nashville. 1996;197-206. Available:http://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=277875
25. Baptista P, Felizardo, Menezes P, Correia JC. Multivariate NIR for predicting the Iodine value, CFPP, KInematic Viscosity@40 and density at 15 degrees. Talanta. 2008;77:144-151. DOI: 10.1016/j
26. Ayhan Demirbas. Bio-diesel: A realistic fuel alternative for diesel engines. 2006;1. DOI: 10.1007/978-1-84628-995-8
27. Knothe G. Structure indices in FA chemistry. How relevant is the iodine value? J. Am. Oil Chem. Soc. 2002;79(9): 847–853.
28. Kyriakidis NB, Katsiloulis T. Calculation of iodine value from measurements of fatty acid methylesters of some oils: Comparison with the relevant American oil chemist's society method. J. Am. Chem. Soc. 2000;77(12):1235–1238.
29. Sahoo PK, Das LM, Babu MKG, Naik SN. Bio-diesel development from high acid value polanga seed oil and performance evaluation in a CI engine. Fuel. 2007; 86(3):448-454. DOI: 10.1016/j.fuel.2006.07.025
30. Mat R, Ling OS, Johari A, Mohamed M. In situ bio-diesel production from residual oil recovered from spent bleaching earth. Bulletin of Chemical Reaction Engineering & Catalysis. 2011;6(1):53-57. DOI: 10.9767/bcrec.6.1.678.53-57

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