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Spectroscopic and Chromatographic Investigation of Pupae Biodiesel

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ABSTRACT: Methyl esters from animal fats/lipids have attracted a great deal of interest as substitute for diesel fuel to reduce dependence on imported petroleum and provide an alternate and sustainable source for fuel with more agreeable environmental properties. In the present study biodiesel is prepared from dead Pupae. Because dead pupae are one such source which is generated as waste byproduct of sericulture industry. The Pupae biodiesel was chemically characterized with analytical techniques like FT-IR, NMR and GC–MS. So Results of study obtained are presented and discussed.

Keywords - FAME, Methanolysis, Pupae oil, Pupae biodiesel, Transesterification.

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I. INTRODUCTION

Production of silk is a common practice in different states of India and it has been in practice in India since time immemorial and has a close link with the tradition and socioeconomic life of the people. Nowadays, sericulture is spreading to different non-traditional states like Andhra Pradesh, Tamil Nadu, Karnataka, Gujarat, Jharkhand and Chhattisgarh. Silkworms are utilized for the production of silk in silk reeling industries. The sericulture activities generate huge quantities of the waste dead silkworm pupae after production of the silk from cocoons. As silkworm pupae contributes 60% of the cocoon weight where it was discarded as waste material after use[1]. In India, about 25,547 tons of raw silk is produced from that 15,328 tons of dead Pupae per annum was produced during year 2018.Most of the waste pupae are used as fertilizer and a small amount is used as poultry feed. The major difficulty in storing the waste pupae is its bad smell and poor shelf life. The dumping of the huge waste of the silk industries poses a threat to the environment.

Previous research studies have reveal that the waste pupae contain 30-35% oil along with many other essential nutrients[2]. Silkworm can be used improve the health benefits of the society. Silkworm pupae oil is considered as a good source of edible oil which can be used in some various applications like food, medicine and cosmetics.

In the present work an investigation of the pupae oil obtained from the waste pupae has been carried out to assess its potential as biodiesel The oil was extracted from the dried pupae powder by using Soxhlet apparatus and its conversion to fatty acid methyl esters (FAME) via a methanolysis with base catalyzed transesterification process [3] was investigated. The transesterified product was examined and some quality parameters were measured to establish its suitability for use as biodiesel. The biodiesel formed was characterized by different physico- chemical properties and its composition was determined by employing various instrumental techniques like FT-IR, NMR and GCMS.

II. MATERIALS AND METHODS

2.1 Materials

Pupae used for present investigationshown in fig.1 were obtained from sericulture unit located at Tolhunase in Davangere district and also from Shirahatti, Gadag district. About 10 kg's of dead pupae was collected for each trial run and the properties of dead pupae are shown in table 1. As Dead pupae contain 130-150% of moisture so it is dried in hot air oven within 3 hours of production to remove 90 % of moisture and make it to dried crispy state. Later it is grinded to fine powder using domestic mixer grinder to preserve in an air tight container for long duration, so pupae powder contains 30-35% of oil in it. Hence Pupae oil extractions studies were carried out with the help of Soxhlet Extractor developed in the institute using n-Hexane as solvent. Four kg of powdered sample in two cotton bags and 10 liters of n-Hexane were used in all trials. Operating temperature is maintained at 70°C for n-Hexane. Extractions were carried out for 15 cycles. Residual powder left

over after oil extraction at extractor bed is taken out and Oil micelle remaining in it is squeezed out in to the container, Later Residual powder allowed to dry in the air. At the end dried pupae powder meal is produced. Oil micelle collected at solvent tank was batch distilled to recover solvent as distillates. Around 3 liters of oil micelle is taken in distillation tank for trail run. Heating of raw pupae oil is carried at 80°C in the distillation unit for one hour. As result pure pupae oil is left over at distilling tank were transferred to a measuring jar. Around 2.5 liters of pupae oil and 0.5 liters of n-Hexane were collected.Finallypupae oil Extracted is shown in fig.2.

Table 1: Properties of Dead Pupae

Fig. 1 Dead Pupae

Appearance	Oval shape non-sticky
Colour	Dark orange
smell	Unpleasant,
Weight	0.6-1.2 gm
Diameter	6-8 mm
Length	12-20 mm
Bulk Density	850-860 kg/m^3
Moisture content	130-150 %

Fig. 2 Pupae oil

2.2 Analytical Methods

The fuel properties of synthesized Pupae biodiesel were determined by ASTM methods. Pupae biodiesel was characterized by FT-IR, using a Bruker BX L118 – 5255. NMR analyses were performed using Bruker(AV III-500) and GCMS studies were carried out by JEOL GC Mate-II GC-MS test rig.

III. EXPERIMENTAL PROCEDURE

3.1 Transesterification

The transesterification was carried out using large capacity round bottom container equipped with reflux condenser, magnetic stirrer, thermometer and sampling outlet. 50 liter Pupae oil was filtered and preheated up to 120 °C to remove moisture on a heating mantle. The transesterification of pupae oil was carried out with 13 liters of methanol and 300grams KOHas catalyst. Pupae oil was transesterified with methanol in the presence of KOH as catalyst. The temperature of the reaction was maintained at 60 °C and the contents were stirred for 1 h. The resultant mixture was cooled to room temperature for the separation of two phases. The upper lighter phase of biodiesel and lower denser phase of glycerin (by-product) were separated by simple decantation.Crude biodiesel contains the excess methanol, unused catalyst, soap formed, some entrained methyl esters and partially reacted glycerides. So biodiesel is washed with water to remove all these impurities and later washed biodiesel is heated to 120°C using electric heater to remove water traces. So finally dark maroon color pure pupae biodiesel obtained is shown in fig.3.
The yield of Pupae biodiesel formed was calculated by using the expression [4],

$$\% \text{ Yield} = \frac{\text{Grams Of Methyl Ester Produced}}{\text{Grams of Oil taken}} \times 100 \qquad \text{(i)}$$

The percentage conversion of triglycerides (pupae oil) to the corresponding methyl esters (pupae biodiesel) by using Eq. (i) was found to be 72%.

Fig.3 Pupae Biodiesel

IV. CHARACTERIZATION OF PUPAE BIODIESEL

The synthesized pupae biodiesel was characterized for its fuel properties i.e., density, kinematic viscosities, cloud point, pour point, flash point and Free fatty acids, employing the methods of American Society for Testing and Materials (ASTM)[5][6] and Oil Technologists Association of India (OTAI)[7]. The results are given in Table 2 along with recommended values for biodiesel (ASTM-D6751) and diesel (ASTM-D975). The determined density of pupae biodiesel at 40 °C, was 880 kg/m^3.Which is comparable to the limits for diesel and biodiesel (Table 2). Viscosity is the most important property of biodiesel since it affects the operation of fuel injection system, particularly at lower temperature when the increase in viscosity affects the fluidity of the fuel. High viscosity leads to poorer atomization of the fuel spray during injection into engine cylinder. The determined

kinematic viscosity of pupae biodiesel at 40 °C was 2.88 mm^2/s, which are within the limits of ASTM D6751 of biodiesel and ASTM D975 values for diesel (Table 2).The pour point (pp) is the temperature at which the amount of wax, out of solution is sufficient to get the fuel to clog, thus it is the lowest temperature at which the fuel can flow whereas cloud point (cP) is the temperature at which wax first becomes visible when the fuel is cooled[4]. The determined values of cP and pp of pupae biodiesel were 12°and 6°C, respectively, which are within the prescribed ASTM limits for diesel fuels (Table 2). The flash point is a parameter which is considered in the handling, storage and safety of fuels and inflammable materials. The observed flash point of pupae biodiesel (103°C) is higher than the limits of ASTM D975 for diesel and within the limit to that of ASTM D6751 values for biodiesel (Table 2). Acid number is the measure of free fatty acids (FFA) in oil as well as in biodiesel. Hence the determination of the free fatty acid in pupae oil/biodiesel was found by titrating against standard NaOH aqueous solution using Isopropyl alcohol mixed with phenolphthalein as an indicator [7].

Table 2: Physical properties of Pupae biodiesel.

Fuel properties	Method used	ASTM D6751 Biodiesel	ASTM D975 Diesel	Pupae Biodiesel
Density (kg/m^3)	ASTM D1298	860–900	834	880
Kinematic Viscosity (40°C)(mm^2/s)	ASTM D445	1.9–6.0	1.9–4.1	2.88
Cloud Point (°C)	ASTM D2500	-3.0 to 12	-15 to 5	12
Pour Point (°C)	ASTM D97	-15 to 16	-35 to -15	6
Flash Point	ASTM D93	100–170	60–80	103
Free fatty acids (%)	OTAI	—	—	3.98

V. RESULT AND DISCUSSION

5.1 Chemical Characteristics of Pupae biodiesel

5.1.1 FT-IR spectroscopy

The esters have two characteristically strong absorption bands arising from methoxy carbonyl and CO stretching[4]. The methoxy carbonyl group in Pupae biodiesel changed from 1709.06 cm^{-1} in oil to a strong band at 1709.64 cm^{-1} in biodiesel. The FT-IR spectra of pupae oiland pupae biodiesel are represented in Figs. 4 and 5, respectively. From the spectrum, it observed that the peak of C=O vibration band at 1709.64 of methoxy carbonyl group having a transit to 1709.06 cm−1 in pupae oil to 1709.64 cm−1 in pupae biodiesel. Biodiesel could be exemplify from the transmittance peak at 1709.64 cm−1 corresponding to C=O stretching and hence the resultant compound was confirmed as methyl ester. In addition to this, the peak at 3009.60 cm−1 was associated to –HC = CH– stretching of methyl ester compound. The vibrational peak that appeared at 2922.70 cm−1 of pupae biodiesel was corresponded to asymmetric CH_3 stretching of weaker band. The asymmetric and symmetric CH_3impairment were observed near 1457.3 cm−1 and 1414.8 cm−1 respectively. The vibrational peak at 931.89 cm−1 was assigned to C-O-C stretching. Actually, esters werecharacterized by the strong retention due to C=O stretching frequency near 1709.64 cm−1 and by the strong retention involving the stretching of C-O near 1242.1 cm−1. From this spectrum, the compound produced was proved as methyl ester (biodiesel).

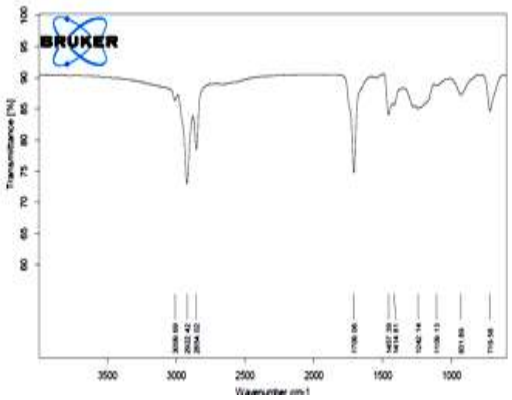

Fig.4 FT-IR spectrum of triglycerides in Pupae oil.

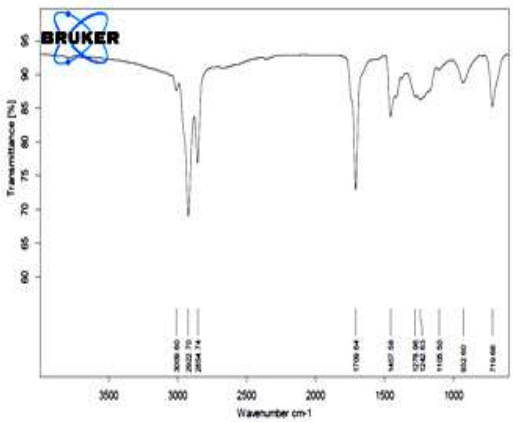

Fig.5 FT-IR spectrum of triglycerides in Pupae Biodiesel.

5.2 NMR spectroscopy

5.2.1 ^{1}H NMR study

The ^{1}H-NMR spectrum of biodiesel sample obtained from pupae oil presented in fig.6 shows characteristic peaks due to olefinic protons (unsaturated –CH=CH-) at 5.3-5.5 ppm (multiplets), very prominent peak at 0.93ppm is due to existence of terminal methyl group, which is considered as the most important structural characteristics of methyl ester. The peaks at 3.35ppm and 1.27ppm were due to protons of ester group (-OCH3, methoxy group) and long alkyl chain (-$(CH_2)_n$). The –CH_2- protons flanked between unsaturated protons (-CH=CH-CH_2-CH=CH-) shows a signal at 2.8ppm. The appearance of methyl groups at 0.88, 0.9 and 0.936 ppm are due to saturated and unsaturated fatty chains. The strong peak at 1.48-1.54ppm associated with alpha-methylene group and the peak at 2.04-2.11ppm indicates the presence of beta-methylene group. The absence of peak at 4.0-4.3ppm is due to the four hydrogens at positions 1 and 3 of the glyceride moiety of the triacylglyceride indicating that all the fatty acids are converted in to methyl ester (biodiesel). This is in good agreement with results obtained by spectral studies.

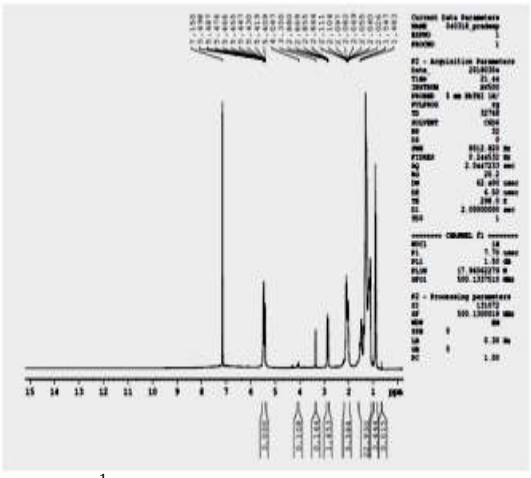

Fig. 6.^{1}H NMR spectrum of Pupae biodiesel (B100)

5.3 Gas chromatography and mass spectrometry

GC–MS was used to study the chemical composition of the synthesized pupae biodiesel. Ten major peaks were observed in GC spectrum of Pupae biodiesel as shown in fig.7. GCMS studies were done to obtain information about number of components, their relative amounts and the size of organic molecules associated [8]. Studies were carried out with the help of JEOL GC Mate-II GC-MS, which had provisions to measure EI (Electron ionization) Mass of 10-1500 AMU range. Mass chromatogram of fig.7 clearly reveals that Pupae biodiesel contains two major fractions. First fraction has a retention time of 20.48 minute, while the second fraction has peaks with retention times of 23.15 minutes. Area of second peak is greater than the area of first peak indicating that second types of fractions are larger than first type. Presence of number of smaller peaks at 18.33 minute, 19.3 minute and at higher retention time intervals at 26.87 minute, 27.35 minute indicates that, the sample also contains number of other ingredients but they are all of trace amounts. Larger retention time indicates that fractions involved are all of high molecular weights. Typical GCMS spectrum of Pupae biodiesel sample obtained is shown in fig.8. Represents the full spectrum of fatty acid profile of pupae biodiesel has the 12 fatty acids screened. Representing the long chainsaturatedfatty acid methyl ester (FAME) suchas C_{11},C_{12},C_{15}, $C_{18}$and also presence of short chain FAME of C_5 to $C_{10}$are Predominant in pupae biodiesel. Relative amount of long chain and short chain FAME compounds are in comparable proportion indicating short ignition delay and possess good anti knocking properties of pupae biodiesel

Spectral studies conducted on biodiesel sample have conclusively shown that

i) Pupae Biodiesel sample mainly consists of mixture of 4 saturated and 8 unsaturated type of FAME compounds by GCMS data.
ii) Two types of unsaturated of very close carbon chain compounds are present.
iii) Few compounds present have long carbon chain type of structures.
iv) All compounds have a methyl ester group at one end of the chain.
v) Size of unsaturated are slightly larger than size of saturates.
vi) There are no branched chains and aromatic substituted types of compounds in pupae biodiesel fuel.

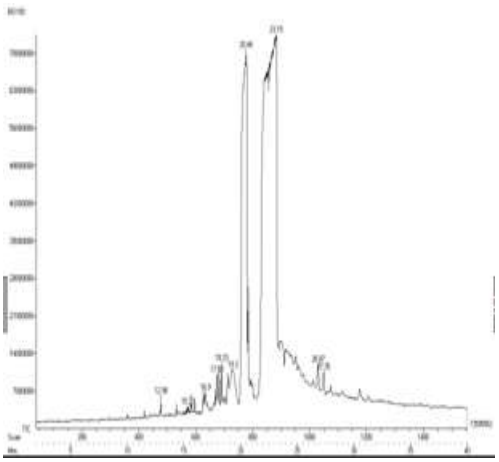

Fig. 7 Mass Fragmentation Spectra of First Ion Peak at 23.15 min.

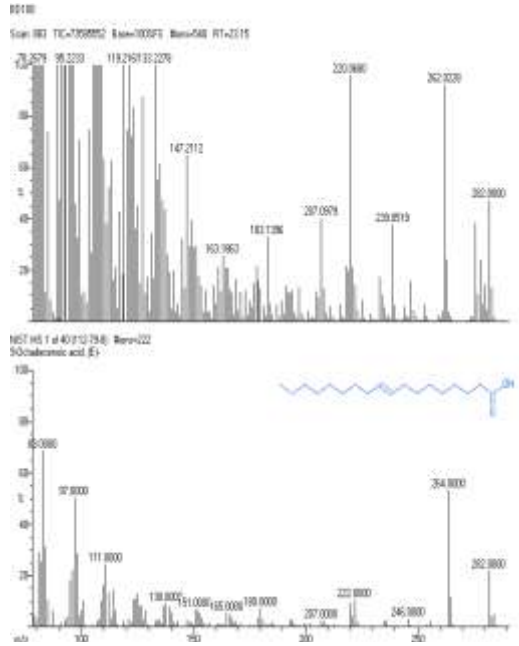

Fig.8 GCMS spectrum of Pupae biodiesel sample.

VI. CONCLUSIONS

Pupae biodiesel was syntheses by base catalyzed transesterification with methanol. kinematic viscosity (2.88 mm^2/s), density ($880kg/m^3$), pour point (6 °C), cloud point (12 °C), flash point (103°C) and FFA (3.98%) of Pupae biodiesel met the ASTM standards after transesterification. Formation of FAMEs was confirmed by FT-IR, NMR and GC–MS analyses. The chemical composition of Pupae biodiesel shows ten types of FAMEs as identified by retention time's data and verified by mass fragmentation pattern. The high percentage conversion of pupae oil into pupae biodiesel indicates that pupae oil has great potential for commercial production of biodiesel.

VII. ACKNOWLEDGEMENT

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