Internationally indexed journal

Indexed in Chemical Abstract Services (USA), Index copernicus, Ulrichs Directory of Periodicals, Google scholar, CABI, DOAJ, PSOAR, EBSCO, Open J gate, Proquest, SCOPUS, EMBASE, etc.

Rapid and Easy Publishing

The “International Journal of Pharma and Bio Sciences” (IJPBS) is an international journal in English published quarterly. The aim of IJPBS is to publish peer reviewed research and review articles rapidly without delay in the developing field of pharmaceutical and biological sciences.

Pharmaceutical Sciences
- Pharmacuetics
- Novel drug delivery system
- Nanotechnology
- Pharmacology
- Pharmacognosy
- Analytical chemistry
- Pharmacy practice
- Pharmacogenomics

- Polymer sciences
- Biomaterial sciences
- Medicinal chemistry
- Natural chemistry
- Biotechnology
- Pharmacoinformatics
- Biopharmaceutics

Biological Sciences
- Biochemistry
- Biotechnology
- Bioinformatics
- Cell biology
- Microbiology
- Molecular biology
- Neurobiology
- Cytology
- Pathology
- Immunobiology

Indexed in Elsevier Bibliographic Database (Scopus and EMBASE)
SCImago Journal Rank 0.129
Impact factor 0.67*

*Instruction to Authors visit www.ijpbs.net
For any Queries, visit “contact” of www.ijpbs.net
PRODUCTION OF BIODIESEL FROM POULTRY WASTE

NAGARAJA Y P*1, VENKATESH H S2 AND VINAY M S3

1 Asst. Professor Department of Biotechnology, Nagarjuna College of Engineering and Technology, Venkatagirikote, Mudugurki, Devanahalli Taluk Bangalore - 562 110 Karnataka, India
2Department of Chemical Engineering, Manipal Institute of Technology, Manipal, Karnataka, India-576 104
3Dept. of Biotechnology, Nagarjuna College of Engineering and Technology, Devanahalli, Bangalore- 562 11 0

ABSTRACT

This paper highlights the significance of biodiesel production as a sustainable potential feedstock from chicken fat by single step transesterification process. Effect of various process variables such as amount of catalyst, temperature, amount of methanol and reaction time on biodiesel production was investigated. The optimal condition for processing (66.7g) of chicken fat was obtained. Under optimal conditions, the maximum yield of (94.76%) chicken fat methyl esters was achieved by using (1:9) molar ratio of chicken fat to methanol, at temperature (35°C), for a reaction time of (45min) in the presence (1%) by weight of Potassium hydroxide(KOH) catalyst. The fuel properties like Density, kinematic viscosity, acid value, Moisture content, iodine value, saponification value, peroxide value, fire point and Flash point was estimated and was found to compare well with ASTM standards. To analyse the FAME, NMR was used and confirmed. Chicken fat was found to be highly suitable to produce biodiesel with recommended fuel properties.

KEYWORDS: Transesterification, poultry waste, Triglycerides, FAME, NMR

*Corresponding author
INTRODUCTION

Biodiesel is an alternative fuel to fossil diesel and its use contributes to attenuate current energy problems. It is renewable, being produced from triglycerides sources, such as vegetable oils and animal fats. Commonly, a transesterification reaction with methanol is performed to produce biodiesel and glycerols as a by-product. Biodiesel can replace fossil fuel as a “clean energy source”. It can protect the environment by reducing CO$_2$, SO$_2$, CO, HC. The carbon cycle of Biodiesel is dynamic through the photosynthesis process. Plants absorb CO$_2$, which is more than those discharged by the biodiesel combustion process. Thus, using biodiesel can more effectively reduce the emission of CO$_2$, protect the natural environment and maintain the ecological balance, compared to the use of fossil fuel. Economic feasibility of biodiesel depends on the availability of low-cost feedstock. As a consequence, animal fats increasingly play an important role to turn biodiesel competitive, mainly in regions with intensive livestock such as southern Brazil, where this material deposition occurs in abundance, with immediate availability, and relative low prices. On average, at present, beef tallow totalizes 17% of feedstock applied in the Brazilian biodiesel production. However, the contribution of animal lipid sources to bioenergy sector is likely to increase considering the accessibility to other profitable raw materials such as chicken and swine fat wastes. The most common feedstock for biodiesel is rapeseed oil in Europe and soybean oil in the United States of America. The major handicap is the high cost of biodiesel for its commercialization. Chicken fat is a low cost feedstock for Biodiesel production compared to high-grade vegetable oils. It is extracted from feather meal which is prepared from chicken wastes such as chicken feathers, blood, offal and trims after rendering process. Feather meal contains significant amount of chicken fat. The fat content of the feather meal varies from 2 to 12% depending on the kind of feathers used. Transesterification is a chemical process of reacting triglycerides with alcohol in the presence of a catalyst. Catalysts can be alkaline, acids, or enzymes (lipases). Compared with acid catalysis, it is 4000 times faster and requires a lower molar ratio of reagents. The purpose of the present study was to produce methyl biodiesel from chicken fat derived from poultry waste and to investigate the effect of fat to methanol molar ratio, temperature, reaction time, and catalyst amount on the yield of biodiesel. In addition, the biodiesel obtained was characterized by determining its physico-chemical properties. The properties were compared well with the ASTM standards. NMR spectroscopy of fat and biodiesel confirmed the conversion of fat to biodiesel and also indicated the completion of reaction.

MATERIALS AND METHODS

Poultry waste was collected from Century Chicken Farms, Manipal, India around September 2012 to November 2012. The chemicals are Methanol (99% pure, AR grade), KOH (86% assay, AR grade), tannic acid (AR grade), stearic acid (AR grade) were purchased from Finar chemicals, India, and were used as received.

Extraction and purification of fat from poultry waste.

The waste was then washed with tap water to remove dirt and grit. The dirt free waste was then cut into small pieces of length 1.5-2.0 inches and stored in air-tight plastic containers for further use. Accurately weighed amount of waste was transferred into a three-necked round bottom flask and double distilled water was added in the ratio 2:1 (v/w). Chicken fat was extracted by heating the above preparation to its boiling point in a heating mantle for a period of 90 minutes. Fat was separated from water using a separating funnel. The bottom water layer was discarded and the top fat layer was collected and stored in air-tight containers and refrigerated at 10$^\circ$C. Acid value of the fat was determined, and the acid value showed...
that the free fatty acid content (FFA) was low. Hence, no pretreatment of raw material (i.e., saponification) was done. The molecular weight of fat was determined using freezing point depression method. The molecular weight was then used to calculate the amount of methanol required for transesterification reaction.

**Transesterification of fat to biodiesel.**
The fat obtained from the above process was heated at 100°C for one hour to remove traces of moisture. Moisture free fat was cooled to room temperature and transferred to a three-necked round bottom flask equipped with a thermometer and a reflux condenser. Desired amount of KOH was dissolved in methanol and was added to the flask containing fat. The reaction mixture was agitated with the aid of a magnetic stirrer at 140 RPM. Temperature was adjusted by varying the power supply to the heating mantle. Optimization of the reaction conditions was done by varying one variable at a time while maintaining the other variables constant.

**Purification and characterization of biodiesel.**
After the transesterification process, the product was transferred to a separating funnel and allowed to stand overnight. The bottom layer (glycerol) was discarded and the top layer was collected in another separating funnel and washed with water followed by a wash with acidified water (0.5 % by weight tannic acid). This was done in order to remove excess methanol and catalyst from the biodiesel. The properties were determined and compared with ASTM standards. The presence of methyl esters and the extent of conversion were confirmed by obtaining NMR (Bruker, Ascend 400MHz) spectra of the product.

**Effect of temperature on yield of FAME.**
The effect of temperature on yield of biodiesel was studied by varying the time of contact of reactants (30, 45, 60, 120, 180, 240 and 300 minutes) while keeping other parameters constant. A molar ratio of 1:9, agitation speed of 140 RPM, a temperature of 35°C, and a 1% catalyst amount were maintained.

**Effect of catalyst concentration.**
Different amounts of catalyst (0.5, 1, 2, 3, and 4 % by weight) were added and the reactions were performed at constant values of other variables. Molar ratio of 1:9, temperature of 35°C, reaction time of 45 minutes and agitation speed of 140 RPM were maintained.

**Effect of fat to methanol molar ratio.**
The effect of molar ratio on yield was studied by varying the volume of methanol added for reaction. The different ratios taken were 1:3, 1:6, 1:9, 1:12, and 1:15. The other variables were maintained constant at a contact time of 45 minutes, a temperature of 35°C, agitation speed of 140 RPM and a catalyst amount of 1% w/w.

**RESULTS AND DISCUSSION**

In the present extraction process, a yield of 6-7% was obtained. A total of 500g of fat was extracted using 7500g of poultry waste. Table 1 shows the physico-chemical properties of fat and biodiesel. The iodine value is used to compare C=C number of natural complex mixtures. The higher values of iodine number indicates the degree of unsaturation. The higher the iodine value higher is the number of double bonds in biodiesel. The lower iodine value gives better biodiesel. Un-saturation in the fatty acid chain is the most significant cause of lower cetane numbers. Iodine values greater than 50 may result in decreased engine life, but give better viscosity characteristics in cooler conditions. Rao, et al., have investigated and found a linear
relationship between NO\textsubscript{x} emissions and the iodine value for seven different methyl esters produced from various oils. Hence, higher the iodine value, higher will be the emissions and lower the cetane number \(^6\). FAME produced from poultry waste fat has an iodine value of 2.284 g I\textsubscript{2}/g of FAME which indicates a lower degree of un-saturation and therefore lower emissions.

### Table -1

**physico-chemical properties of biodiesel and fat.**

<table>
<thead>
<tr>
<th>Property</th>
<th>FAME from poultry waste</th>
<th>ASTM Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinematic viscosity at 30\textdegree C (cst)</td>
<td>2.1</td>
<td>1.9-6.0</td>
</tr>
<tr>
<td>Density at 30\textdegree C (kg/m\textsuperscript{3})</td>
<td>868</td>
<td>878-885</td>
</tr>
<tr>
<td>Acid Value (mg KOH/g FAME)</td>
<td>0.8041</td>
<td>&lt;0.8</td>
</tr>
<tr>
<td>FFA content of fat (%)</td>
<td>0.28</td>
<td>-</td>
</tr>
<tr>
<td>Iodine Value (g/100g)</td>
<td>2.284</td>
<td>115</td>
</tr>
<tr>
<td>Saponification value (mg/g)</td>
<td>590.4525</td>
<td>-</td>
</tr>
<tr>
<td>Moisture content of fat (%)</td>
<td>0.77</td>
<td>-</td>
</tr>
<tr>
<td>Peroxide value (meq/kgFAME)</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Flash Point(\textdegree C)</td>
<td>120</td>
<td>&gt;130</td>
</tr>
<tr>
<td>Fire point (\textdegree C)</td>
<td>160</td>
<td>-</td>
</tr>
<tr>
<td>Molecular weight of fat (g/gmol)</td>
<td>667.667</td>
<td>-</td>
</tr>
</tbody>
</table>

Saponification value is a measure of the average molecular weight of fat or a measure of chain length of the fatty acids present. Based on the length of the fatty acid molecule, the weight of the triacylglycerol molecule changes, which in-turn changes the amount of KOH required for saponification of 1g of fat. Most fatty acids have a saponification value in the range of 185-210 mg KOH/g of fat indicating fatty acids with chain lengths between C16-C18. Higher saponification values may indicate the presence of shorter chain lengths of C12-C14 \(^7\). In this study, the saponification value was found to be 590mg KOH/g of FAME, which may be indicative of presence of shorter length fatty acids. Moisture content of fat plays an important role in trans-esterification of fat to FAME. Moisture in feed-stock may react with catalyst during the production of FAME and lead to formation of soaps and emulsions \(^7\). This poses problems in separation and may also lead to loss of yield. The moisture content of poultry waste fat was found to be 0.77 %, which is negligible. Hence, no further steps were taken to remove moisture and the fat was stored as soon as it was extracted into air-tight containers. The peroxide value gives us an idea about the oxidative stability of the bio-diesel. It is measured in milli-equivalents of peroxide per kg of FAME. Zuleta, \textit{et al.}, reported that an increase in the value of peroxide number of bio-diesel increases its cetane number, thereby reducing the ignition delay. This is explained by the fact that formation of oxides on oxidation are more reactive and accelerate combustion \(^8\). On the contrary, Lin \textit{et al} reported a decrease in cetane number of palm oil bio-diesel with increase in peroxide value. In their defense, they argue that formation of water during oxidation causes ignition delay \(^9\). In this present investigation the peroxide value was calculated to be 9 meq/kg FAME.

The flash point is the lowest temperature at which the fuel emits enough vapors to ignite. Bio-diesel has a high flash point; usually more than 150\textdegree C, while conventional diesel fuel has a flash point of 55-66\textdegree C. If methanol, with its flash point of 12\textdegree C is present in the biodiesel the
flash point can be lowered considerably. Bio-
diesel with low flash points can cause storage
problems and create a potential fire hazard. Bio-
diesel with flash point lower than 93°C is
considered to be out of specifications. Sanford,
et al., reported flash points for bio-diesels from
various sources and none of them were found
to have flash points below 93°C. The least was
cocnut oil (115°C) and the authors reason out
that it’s not due to presence of methanol but
due to the presence of short length fatty acid
chains, that of C12 and C14. Hence, a flash
point of 120°C doesn’t indicate presence of
residual methanol and a value higher than 93°C
indicates a presence of chain lengths higher
than C12-C14. Catalyst (1 wt%), temperature
(35°C), reaction time (45 minutes), and fat to
methanol molar ratio (1:9) were found to be the
optimum conditions for maximum yield of
biodiesel. Figures 1 showed the effects of the
above variables on the yield of biodiesel. Under
optimum conditions the yield of biodiesel was
found to be 94.02%. Moreira et al., report that
most publications on poultry waste as a source
for bio-diesel production have stated that transesterification reaction should be processed
between 60-70°C and only Reddy et al., have
performed this reaction at 25°C. However, at
that temperature they found that the reaction
time required was much longer (6-7 h) and large
quantity of methanol (1:72) was required for
higher yields (1). Eevera et al., have stated
that at elevated temperatures saponification
reaction is favored over transesterification. This
may the cause for decreasing yield with
increase in temperature. Moreover, trans-
esterification being a reversible reaction, follows
Le-Chatelier’s principle and favors backward
reaction at higher temperatures. Marchetti et
al., have stated that base-catalyzed transesterification is exothermic in nature, hence the above reasoning is conclusive.

The highest yield was obtained at 45 minutes.
The yield at 30 minutes is lower than that at 45
minutes because the reaction proceeds slowly
in the initial stages; which may be due to mass
transfer resistance (mixing between oil and
methanol). The yield continues to increase with
increasing reaction times, but as the product
congestion increases the equilibrium shifts
backwards to nullify the effect of this constraint.
(Le Chateliers principle). Therefore it can be
seen that after 45 minutes the yield slowly
decreases and saponification of triglycerides is
favoured. This point of view is supported in a
publication by Mathiyazhagan et al.,.
The optimum catalyst amount was found to be 1\% w/w. Catalyst amount lower than 1\% proves to have a negative impact on the yield because of decrease in active sites available for acceleration of reaction. From figure 3.3 it can be seen that between 1-1.5\% concentration of the catalyst the yield is almost constant and then starts to decrease steeply. This is due to the fact that higher concentration of KOH leads to saponification of tri-glycerides. Also, according to Mathiyazhagan et al, formation of water when the catalyst is added inhibits trans-esterification and instead favours hydrolysis (10). This is the reason for dissolving the catalyst in methanol first and then transferring the mixture to fat.

Molar ratio of alcohol plays a very important role in production of bio-diesel\textsuperscript{10}. Stoichiometrically, three moles of methanol is required to esterify triglycerides. However, according to Le Chatelier a higher concentration of one of the reactants shifts the equilibrium forward. Therefore an increase in yield with molar ratio is seen. In this study, the optimum molar ratio was found to be 1:9. At ratios higher than 1:9, the yield seemed to decrease slightly. Liu et al report that high alcohol to fat molar ratios slow down the reaction owing to catalyst concentration diminution by large excess of alcohol\textsuperscript{12}.
Figure 4

Effect of molar ratio on yield.

Figure 5 displays $^1$H-NMR spectra of the FAME sample. The peaks at 5.35 ppm, 2.8 ppm and 2.1 ppm are related to the $^1$H located at or near the double bond(s) within the unsaturated methyl esters, 18:1, 18:2, and 18:3. The sharp peak at 3.7 ppm is due to the ester methyl located next to the carbonyl carbon and the triplets around 0.9 ppm are from the terminal alkyl methyl in each of the methyl esters. The methylene alpha to the ester group is at 2.3 ppm and the beta group is at 1.6 ppm. The remaining CH2 group protons have similar resonance frequencies and overlap in the range of 1.2–1.4 ppm$^{13}$. 

Figure 5

NMR spectrum of biodiesel from poultry fat
Figure 6 shows spectra for fat from poultry waste fat. It shows a multiplet at 4.1 ppm, which is due to the presence of triglycerides. The other hydrocarbon peaks are at their normal positions. The disappearance of this peak in the spectrum of bio-diesel and appearance of a peak at 3.662 ppm confirms the conversion of fat to bio-diesel. The spectral data have also been used to evaluate the completion of reaction. The spectral data was also used to find the degree of un-saturation present in the sample. The degree of un-saturation was found to be 3.958.

**ACKNOWLEDGEMENT**

We acknowledge to Centaury Chicken Farm, Manipal, India, for providing feather meal samples. Authors acknowledge to instrumentation department MIT for providing NMR Spectroscopy. Finally our special thanks to C R Girish and Anup Sarovar (MIT,Manipal,India).

**REFERENCES**


