Volume 3 | Issue 2, 2023 | Pages 59 - 65

KRISTU JAYANTI JOURNAL OF CORE & APPLIED BIOLOGY

ORIGINAL RESEARCH

Biodiesel Production from Fish Waste of *Ctenopharyngodon idella* (Grass fish), *Oreochromis niloticus* (Tilapia) and *Sardina pilchardus* (Sardine)

N. Mahalakshmi[1], Sangeetha Menon[2*], P. Senbagalakshmi[3], T.Thiruselvi[1] and A. Xavier Fernandes[1]

[1]*Department of Microbiology, School of Arts & Science, Ponnaiyah Ramajayam Institute of Science & Technology (PRIST), Thanjavur, Tamil Nadu*
[2]*Department of Life Sciences, Kristu Jayanti College Autonomous, Bengaluru*
[3]*Department of Botany, A.D.M College for Women (Autonomous), Nagappatinam, Tamil Nadu*

Abstract

Numerous tons of fish waste are annually produced by fish processing facilities, leading to significant environmental issues related to emissions and degradation. Nonetheless, a viable solution exists in the conversion of these wastes into valuable resources such as biofuels, pharmaceutical ingredients, fertilizers, and animal feed. Among various waste sources, fish waste emerges as an optimal raw material for the production of biodiesel. In this research, fish waste from three distinct species viz., Ctenopharyngodon idella (grass fish), Oreochromis niloticus (tilapia), and Sardina pilchardus (sardine), were utilized as substrates. The wet extraction method yielded 92.15% FW oil. Physicochemical analysis revealed 3.487 cm-1 free fatty acids and an acid value of 7,291 cm-1 in FW oil. Post cross-esterification, the free fatty acid content reduced from 2.543 mg/KOH/Kg to 0.944 mg/KOH/Kg, while the acid value dropped from 6.452 mg/KOH/Kg to 0.839 mg/KOH/Kg in biodiesel production. Spectral analysis (FTIR) identified a prominent peak indicating the presence of a methyl group (CH3) or methylene group in both crude FW and biodiesel samples. The moisture content of FW crude oil (0.69%) and biodiesel (0.00%) confirmed the absence of water post cross-esterification process completion. Ultimately, transesterification of FW crude oil utilizing a heterogeneous catalyst derived from fish waste is deemed an economically efficient approach for biodiesel manufacturing purposes.

Keywords

Fish waste, FW oil, biodiesel, transesterification, free fatty acid

Article History
Received: 21 June 2023
Revised: 22 September 2023
Accepted: 24 September 2023
Published online: 29 December 2023

Author for Correspondence
sangeethamenon@kristujayanti.com

1. Introduction:

Energy is widely acknowledged as a pivotal element in the economic advancement of nations. Fossil resources, including coal, natural gas, and crude oil, play a significant role in fulfilling the energy demands of major nations. With the rapid pace of industrialization and urbanization, global energy consumption is forecasted to rise by 33% by 2035 *(Esmi et al., 2022; Bateni et al., 2017)*. The depletion of non-renewable resources and their detrimental effects on environmental issues such as global warming have spurred efforts towards identifying alternative methods for conserving energy and electricity *(Ong et al., 2021; Khan et al., 2022)*. Strategies focusing on energy efficiency and environmentally sustainable energy production have garnered international attention *(Jayed et al., 2009)*. Biodiesel refers to long-chain fatty acid monoalkyl esters derived from renewable sources like vegetable oils or animal fats *(Charpe and Rathod, 2011; Knothe, 2010)*. Apart from triglyceride-based biomass such as soybean, palm, castor, and rapeseed oils, biodiesel can also be synthesized from animal fats like lard, chicken fat, and fish oil capsules *(Wisniewski et al., 2010; Karkal and Kudre, 2020)*. The fish processing industry generates substantial amounts of tissues and by-products that are often discarded as fertilizers or animal feed or sold at minimal prices *(Behcet, 2011)*.

Animal fat-containing feedstocks identified and considered for biodiesel production include fish waste, which attracts strong attention because it forms a significant amount of by-products such as bones, scales, organs, etc. *(Ghaly et al., 2013)*. This approach can ensure the availability of raw materials for the local

Cite this article as: *N. Mahalakshmi, Sangeetha M, P. Senbagalakshmi, T. Thiruselvi1 and AX Fernandes (2023). Biodiesel Production from Fish Waste of Ctenopharyngodon idella (Grass fish), Oreochromis niloticus (Tilapia) and Sardina pilchardus (Sardine), Kristu Jayanti Journal of Core and Applied Biology. 3(2):59-65*

conversion of biodiesel which classically uses homogeneous catalysts due to their high catalytic activity *(Karkal and Kudre, 2021, 2023)*. However, several challenges with homogeneous catalysts that require the search for suitable substitutes include nonreusable reactions and saponification in the presence of moisture *(Chen et al., 2015)*. In this case, heterogeneous catalysts are used to overcome the disadvantages of homogeneous catalysts: they are environmentally friendly, non-corrosive and can be synthesized from biomass and non-biomass sources *(Ling et al., 2019)*. *Piloto-Rodríguez et al. (2013)* reported that reduced reaction time, economic viability, and improved biodiesel conversion rate are some of the advantages of heterogeneous catalysts over their homogeneous counterparts.

In this study, biodiesel production from crude fish waste (FW) oil was examined using the transesterification method.

2. Materials and Methods:

2.1. Collection and processing of fish waste samples:

Three distinct types of fish waste from *Ctenopharyngodon idella* (Grass fish), *Oreochromis niloticus* (Tilapia) and *Sardina pilchardus* (Sardine), was collected from the neighborhood fish market, Karaikudi, Tamil Nadu. The gallbladder, fish vesicles, and intestinal contents were segregated from the fish waste samples and were washed three times with tap water. These samples were blotted using filter paper and then freeze-dried for 6-7 hrs. After the dehydration process, these samples were homogenized in a homogenizer, while a small amount of tough tissue, such as the intestinal tissue, which could not be completely homogenized, was discarded via a large-hole screen. The remaining homogenate biomass was evenly divided into several small containers and stored at -20˚C until use.

2.2. Sample Pre-Treatment :

To ensure successful extraction of oil, the fish waste samples were pretreated as described by *Demirbas et al. (2016).* The fish waste samples were washed using distilled water several times to remove dirt and the remaining blood. Then the samples were dried in a hot air oven at 55˚C - 60˚C till complete dryness. The dried sample was crushed into powder form in a mortar pestle and stored in sealed plastic bags at 4˚C until use.

2.3. Extraction of Fish Waste Oil (FWO):

The Fish Waste Oil (FWO) was extracted with petroleum ether using Soxhlet TM 2043. Extraction was done based on the manual of Soxhlet TM 2043, where 60˚C temperature was maintained. The extracted crude fish oil was then kept in the laboratory chiller for further analysis.

2.4. Biodiesel Production:

2.4.1 Pretreatment of FWO

Pretreatment of FWO was carried out by method described by *Agu et al. (2024)*. The FWO was filtered in vacuum to remove impurities and water. It was then degummed with phosphoric acid and water to remove polar components such as phospholipids, lecithin, pigments, and contaminants such as heavy metals. Neutralization was accomplished by adding potassium hydroxide to remove free fatty acids from the soap. Silica gel column chromatography was used to remove impurities and colors using (90% cyclohexane and 10% ethyl acetate) as the solvent. It was then easily dried by heating at 1050C for 15 minutes. Finally, deodorization was carried out by vacuum heating.

2.4.2 Transesterification Process

Neutralization of FWO was carried out using 5N Potassium hydroxide (KOH). The resulting mixture was heated at a temperature of 110°C for a duration of 15 minutes to eliminate any residual moisture. Subsequently, the transesterification process was conducted within a sealed Erlenmeyer flask with a volume of 250 ml, containing a reaction mixture of 150 ml. This flask was positioned in a temperature-controlled water bath and placed on a magnetic stirrer with a consistent stirring rate set at 200 rpm. Following a reaction period of 30 minutes, the catalyst was separated from the mixture through centrifugation at 6000 rpm for 15 minutes. Post-centrifugation, the supernatant comprising glycerol (lower phase) and biodiesel (upper phase) were segregated using a separating funnel. To eliminate any remnants of unreacted catalyst, methanol, and glycerol, the biodiesel underwent washing with 0.5 M citric acid for half an hour, followed by an additional heating step lasting ten minutes to ensure thorough drying of any remaining moisture. The citric acid residue was separated via centrifugation at 4000 rpm for 7 minutes. The biodiesel layer was then collected and its yield calculated using the formula:

$$\text{Biodiesel Yield (wt\%)} = \frac{\text{Weight of Biodiesel (methyl ester)}}{\text{Weight of FWO}} \times 100$$

2.5. Physicochemical Properties of FWO and Biodiesel:

2.5.1 Oil yield

The total oil content of oil extract was determined by using AOAC, (2000) method. Chloroform: Methanol (1:1) was used for oil extraction in Soxhlet apparatus. The cover was introduced into the extractor, and the oil extraction was performed for 2 hours at 60˚C. The solvent was recovered under separation by using a rotary evaporator (RVO 400) at 65C. The oil residue was allowed to dry and the mass was recorded. The total oil content was calculated as *(Tilamia et al.. 2018)*.

$$\text{Oil yield \%} = \frac{\text{Weight of residue}}{\text{Weight of the sample}} \text{ X100}$$

2.5.2 Moisture content determination

The moisture content of oil samples before and after heating was determined by the Association of Official

Analytical Chemists (AOAC, 2004) method. Firstly, the weight of the previously dried (1 hr at 100ºC) crucible with cover was taken and 5-15 g of sample was placed on it. The samples were dried to constant weights in an oven at 105°C, cooled in desiccators and weighed. Drying, cooling and weighing were repeated until the two consecutive weights were the same. From these weights the percentage of moisture was calculated as follows:

$$\text{Moisture \%} = \frac{\text{Initial Weight (g)- Final Weight(g)}}{\text{Weight of the sample(g)}} \text{X100}$$

2.5.3 Density value

The density value of fish oil was determined by(AOAC, 2004) weighing the mass in gram with relative to the known volume of sample in triplicate reading as follows: 10 ml of Sample 1 was taken and weighed to weigh 12.8 g; again same volume and sample weigh 9.3 g and third reading of 9.7 g. While test 2 of same sample with same volume of 10 ml weigh 8.6, 8.25 and 9.1 while third reading is 10.7 g, 9.6 g and 9.47 g based this mass and volume, density value determined as: Mean average of three mass values versus volume is 31.8 g, 25.95 and 30.01 g. Based this mass and volume, density value determined as:

$$\text{Density value} = \frac{\text{Mass(g)}}{\text{Volume(ml)}}$$

2.5.4 Acid value measurement

The acid value was determined by the ISO 660:1996 (International Organization of Standardization, 1996) method. Approximately 2 g of oil was mixed with 10 ml ethanol, followed by two drops of phenolphthalein. The Combination was boiled for about five mins and then titrated with 0.1N KOH till a pale pink color seemed. The acid value was calculated using the following equation:

$$\text{Acid value} = \frac{\text{Titre X N of KOH X 56.1}}{\text{Weight of the sample (g)}} \text{X100}$$

2.5.5 Free fatty acid determination

By titrating the alcoholic solution of the oils with an aqueous solution of sodium hydroxide using phenolphthalein indicator, the free fatty acid concentration was determined *(Aletor et al., 1990).* Approximately 10g of the oil was assessed into the conical flask. 50 ml of alcohol ether mixture in equal volume was added and it was warmed in a laboratory hotplate stirrer to obtain a homogeneous mixture. 1 mL of phenolphthalein indicator was then added and was titrated with 0.1N NaOH until a fairly pink endpoint was obtained.

$$\text{Free fatty Acid value} = \frac{\text{Titre (ml) of NaOH X N of NaOH X 28.2}}{\text{Weight of the sample (g)}}$$

2.5.6 Saponification value measurement

Saponification value was determined by the Association of Official Analytical Chemists (AOAC, 2005). Typically, 4 g of oil sample was taken in 250 mL conical flask and added with 50 mL alcoholic KOH and mixed thoroughly. A condenser was fitted to the conical flask and the mixture was heated between 60 and 70oC for an hr. The hot mixture was titrated with 0.5 N HCl using 2 drops of 1% phenolphthalein indicator until a colorless solution was obtained. A blank titration was also accompanied side by side. The saponification value was calculated using the following formula:

$$\text{Saponification value} = \frac{\text{(B-S) X N X 56.1}}{\text{Weight of the sample (g)}}$$

Where B = HCl (mL) for blank, S = HCl (mL) for sample and N = Normality of HCl

2.6 Fourier Transform Infrared Spectroscopy (FTIR) analysis of FWO and Biodiesel:

Both the FWO and the biodiesel derived from FWO were subjected to evaluation via infrared spectroscopy to analyze the composition of functional groups present in these samples. . The spectrum pattern was recorded in transmission (%) versus wavelength (cm^{-1}) mode in the ranges of 3500 – 1000 cm^{-1} *Agu et al. (2024).*

3. Results & Discussions:

3.1 Physicochemical Characteristics of FWO and Biodiesel:

The physicochemical attributes of the FWO *(Table 1)* and the resulting biodiesel derived from FWO *(Table 2)* were evaluated. The extraction technique employed was wet reduction, as documented by *Agu et al. (2024)*, who achieved a yield of approximately 96.85% from catfish waste oil (CFW). Similarly, Karlkal and Kudre (2021) obtained a yield of about 95.2% by weight from marine fish waste (MFW). The moisture content percentage in the FW oil was found to be 0.69%, aligning closely with the findings reported by *de Medeiros et al,. (2019)* for fish waste residue utilized in biodiesel production under extraction conditions of 60°C and 120 minutes. Furthermore, a lower moisture content value of 0.073% was previously recorded by *Agu et al. (2024)* for CFW oil, while *Karlkal and Kudre (2021)* reported a moisture content of 0.14 wt% for MFW, both demonstrating lower values compared to the present study's results.

The free fatty acid content (3.487) and acid value (7.291) of FW oil demonstrate a correlation with the free fatty acid content (3.593 mgKOH/Kg) and acid value (7.186 mgKOH/Kg) previously documented by *Agu et al,. (2024)* for use in CFW oil.However, these findings were juxtaposed with the reported values for fatty acids and free fatty acids in MFW extracted oil, which stood at 6.07 mgKOH/g and 3.1%, respectively *(Karlkal and Kudre, 2021).* These outcomes suggest that further optimization

or esterification processes are required prior to transesterification.

The saponification value of CFW oil (295.557 mg/KOH/Kg) surpasses the value of 295.557 mg/KOH/Kg put forth by *Agu et al. (2024)*. Fish oil exhibits a saponification value of 196.35 mg/g *(Santya et al., 2019)*, indicating a high triglyceride content suitable as raw material for biodiesel production. The viscosity of CFW oil (48,782 mm2s·1 @ 40°C) is relevant to diesel engine atomization processes and aligns with the viscosity of MFW at 43 cP @ 25°C according to *Karlkal and Kudre (2021)*. Nevertheless, these values exceed the ASTM's recommended range of 1.9 to 4.1, signifying that further processing through transesterification is imperative to evaluate its viability as a biodiesel feedstock material.

Table 2 presents the physicochemical characteristics of biodiesel derived from FW crude oil through a transesterification process. Certain parameters in *Table 2* are specific to biodiesel and serve as criteria for assessing the quality of the transesterified oil, as well as for comparing it with previously documented models and studies on biodiesel. Following the transesterification procedure, there is a reduction in the levels of free fatty acids and acid value in the biodiesel produced from crude oil compared to the initial crude oil (as indicated in *Table 1*). This underscores the significant role that transesterification plays in the production of biodiesel. Kinematic viscosity assumes critical importance in overseeing the atomization process within diesel engines owing to insufficient lubrication. Inadequate viscosity can lead to leaks, whereas overly viscous oil may result in incomplete combustion *(Bhuiya et al., 2016; Balat et al., 2011)*. Consequently, regulatory bodies and design standards generally establish acceptable viscosity ranges and standards from this viewpoint.

Table 1: *Physiochemical properties of Extracted oil from FW*

S.No	Parameters	Values
1	Oil yield (%)	92.15
2	Moisture (%)	0.69
3	Density (Kg/m3)	749.6
4	Molecular weight (g/mol)	589.375
5	Saponification (mgKOH/kg)	303.372
6	Free fatty acid (mgKOH/kg)	3.487
7	Acid value (mgKOH/kg)	7.291

Table 2 : *Physiochemical properties of Biodiesel from FW*

S.No	Parameters	Values
1	Free fatty acid (mgKOH/kg)	2.543
2	Acid value (mgKOH/kg)	6.452
3	Moisture (%)	0.00
4	Density (Kg/m3)	702.5

3.2. *Fourier Transform Infrared Spectroscopy (FTIR) analysis of FWO and Biodiesel*

The FTIR spectra patterns of the original (a) and biodiesel (b) derived from FW oil are displayed in *Figures 1 and 2*. The FTIR spectroscopic analysis was utilized to comprehend potential alterations in functional groups and the tentative assignment of peaks in both the original and resultant biodiesel from FW oil, as outlined in *Table 3*. As illustrated in *Figures 1* and *2*, prominent peaks at 2922.38 and 2853.62 cm^{-1} were observed in the original FW oil sample. Similarly, corresponding peaks at 2922.61 and 2854.44 cm^{-1} were identified in the biodiesel derived from FW oil, attributed to the methyl (CH3) or methylene group within ester compounds. Additionally, the absorption peak at 1744.4 cm^{-1} presents in the original FW oil shifted to 1736.9 cm^{-1} in the biodiesel, signifying C=O stretching characteristic of carbonyl esters. Furthermore, peaks at 1459.94 cm^{-1} in the original FW oil transitioned to values of 1457.11 and 1564.54 cm^{-1} in the biodiesel, indicating C=H stretching within methyl esters. The peaks observed at 1157.43 and 1168.99 cm^{-1} in both the original FW oil and biodiesel were associated with stretching vibrations of -C-O- ester groups. Lastly, overlapping methylene –CH3 functionalities were denoted by peaks located at 721.18 cm^{-1}, consistent across both the original FW oil and its resulting biodiesel products.

The FTIR spectra patterns of the raw (a) and biodiesel (b) derived from FW oil are illustrated in *Figures 1* and *2*. The analysis employed FTIR spectroscopy to elucidate potential alterations in functional groups and to provisionally assign peak values for both the raw material and the resulting biodiesel from FW oil (*Table 3*). Noteworthy peaks at 2922.38 and 2922.61 cm^{-1} were evident in both the raw oil and biodiesel derived from FW oil, indicating distinctive vibrational attributes of methyl (CH3) present in the ester chain of biodiesel as noted by previous studies *(Fadhil et al., 2015; de Medeiros et al., 2019; Anand et al., 2015).*

Moreover, an absorption peak at 1743.89 cm^{-1} observed in the raw FW oil shifted to 1741.19 cm^{-1} in the biodiesel,

signifying the C = O stretching of carbonyl ester functionality. This resonance was also identified by Fadhil et al. (2015) at a similar frequency of 1751 cm^{-1} in Cyprinus carpio fish oil, attributing it to the C = O stretching vibration of carbonyl ester. Similarly, *de Medeiros et al., (2019)* recognized a comparable peak at 1740 cm^{-1} in fish waste oil residue associated with the C = O stretching of carbonyl groups.

The peaks detected at 1459.94cm^{-1} in the raw oil and within a range of 1564.54 – 1457.11cm^{-1} in biodiesel corresponded to characteristic vibrations of –CH groupfound in methyl esters, underscoring the conversion process from FW oil triglycerides to fatty acid methyl esters.

Subsequent to transesterification, new peaks emerged at 1036.2 and 857.3 cm^{-1} specifically in FW biodiesel that were absent in the raw material, attributed to O-CH3 stretching effects as documented by *Kudre et al. (2017)*. The methylene –CH3 overlap was attributed to the peaks at 721.18 cm^{-1} and 720.65 cm^{-1}, which were detected in both the unprocessed and biodiesel forms of FW oil.

Table 3: **Peak and spectroscopic assignments of the FWO and biodiesel**

S.No	Wavenumber (cm-1)		Peak assignments
	FWO	Biodiesel	
1	2922.38	2922.61	methyl (CH3) or methylene group in ester
2	2853.62	2922.61	methyl (CH3) or methylene group in ester
3	1743.89	1741.19	C = O Carbonyl group
4	-	1564.54	C–H stretching of methyl ester
5	1459.94	1457.11	C–H stretching of methyl ester
6	1157.43	1168.99	-C-O- ester group stretching
7	1157.43	720.65	-C-O- ester group stretching

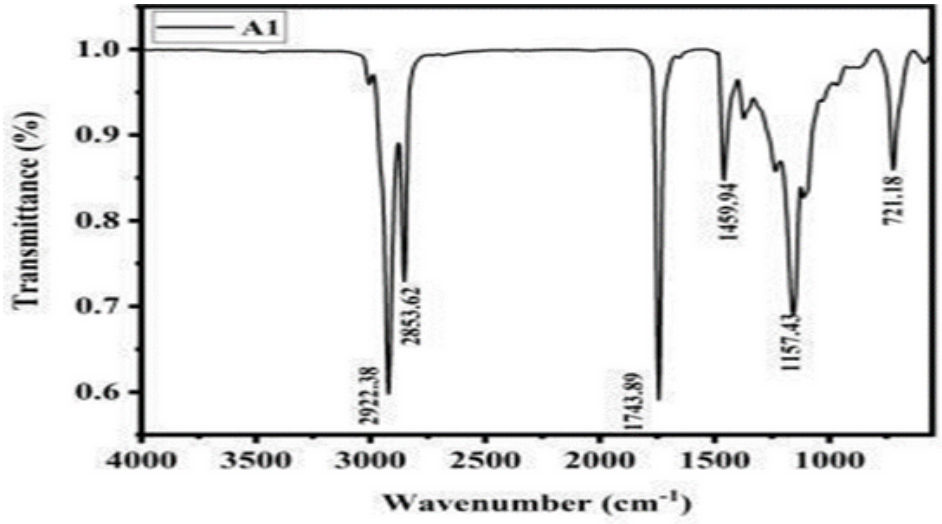

Fig. 1 : **FTIR spectroscopic analysis of FWO**

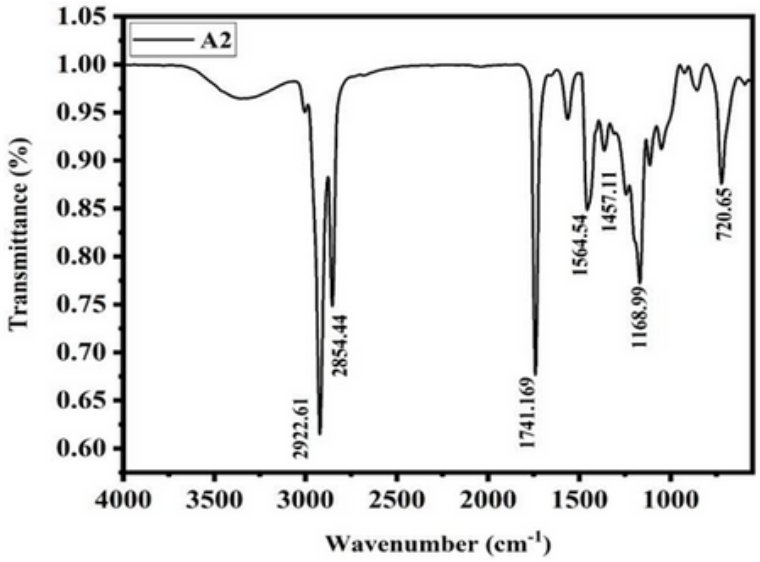

Fig. 2 : **FTIR spectroscopic analysis of Biodiesel from FWO**

4. Conclusion:

In the present study, fish waste used as feedstock for biodiesel production is expected to solve the problem of high concentrations of FFA, water and other impurities. The physicochemical properties of FW crude oil highlight the necessity of transesterification. However, the produced biodiesel has improved physicochemical properties but requires additional processing to meet the requirements of conventional biodiesel standards. The appearance of stretching vibrations of the ester group, observed from the FTIR results, is indicative of the conversion of FW crude oil to biodiesel. Therefore, local biodiesel companies can exploit the potential of FW oil as a suitable low-cost feedstock and help reduce the threat of fish waste disposal problems.

Acknowledgments:

The authors express their sincere gratitude to the Department, Faculty of Fisheries Sciences, Karaikudi, Alagappa University, Tamil Nadu for their invaluable and decisive support and providing the facilities during the conduct of this work.

References:

Agu CM, Ani KA, Abiazieije PO, Omeje JA, Ekuma JC, Umelo UE, Omukwu OH, Nwankwo ED, Chinedu MP (2024). Biodiesel production from waste cat fish oil using heterogeneous catalyst from cat fish born: A viable waste management approach, and ANN modeling of biodiesel yield. Waste Management Bulletin. 1: 172–181

Agu CM, Ani KA, Ani ON, Nnaji PC, Kadurumba CH, Esonye C (2023). Application of efficient soft computing approaches for modeling methyl ester yield from Azadirachta Indica (Neem) seed oil: A comparative study of RSM, ANN and ANFIS. Green Technol. Sustain. 4, 100057.

Aletor VA, Ogunyemi O (1990). The performance, hematology, scrum constituents and economics of producing weaner-pigs on dried brewer's grain. Nigerian Journal of Technology Research, 2(2), 85-89.

Anand M, Gobalakrishnan M, Maruthupandy M, Suresh S (2015). Biodiesel production from mixed crude oil of non-edible marine fishes. Energy Environ. Focus 4(1) (2015) 47-53.688.

AOAC (Association of Official Analytical Chemistry) (1990). Official Methods of the Association of Official Analytical Chemistry. Washington D.C., USA: AOAC.

AOAC (Association of Official Analytical Chemistry) (2004). Official Methods of the Association of Official Analytical Chemistry, 15th ed. Washington D.C., USA: AOAC.

AOAC (Association of Official Analytical Chemistry) (2005). Official Methods of Analysis of Association.

ASTM D1298 (2023). Standard Test Method for Density, Relative Density, or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method.

ASTM D2709 (2022) Standard Test Method for Water and Sediment in Middle Distillate Fuels by Centrifuge.

ASTM D445 (2011). Standard Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (and Calculation of Dynamic Viscosity)

Balat M (2011). Potential alternatives to edible oils for biodiesel production–A review of current work. Energy Convers. Manage. 52, 1479–1492.
olysis: Chemical composition. Fuel 89(3), 563-568.

Bateni H, Saraeian A, Able C (2017). A comprehensive review on biodiesel purification and upgrading. Biofuel Res. J. 4(3), 668-690.

Behçet R (2011). Performance and emission study of waste anchovy fish biodiesel in a diesel engine. Fuel Processing Technology 92(6), 1187-1194.

Bhuiya M, Rasul M, Khan M (2016). Prospects of 2nd generation biodiesel as a sustainable fuel–Part 2: properties, performance and emission characteristics. Renew Sustain. Energy Review 55, 1129–1146.

Charpe TW, Rathod VK (2011). Biodiesel production using waste frying oil. Waste Manage. 31(1), 85-90.

Chen G, Shan R, Shi J, Liu C, Yan B (2015). Biodiesel production from palm oil using active and stable K doped hydroxyapatite catalysts. Energy Convers. Manage. 98 (686), 463–469.

Esmi F, Borugadda VB, Dalai AK (2022). Heteropoly acids as supported solid acid catalysts for sustainable biodiesel production using vegetable oils: A review. Catalysis Today 404, 19-34.

Fadhil AB, Al-Tikrity ETB, Albadree MA (2015). Transesterification of a novel feedstock, Cyprinus carpio fish oil: Influence of co-solvent and characterization of biodiesel. Fuel 162, 215–223.

Ghaly AE, Ramakrishnan VV, Brooks MS, Budge AM, Dave D (2013). Fish processing wastes as a potential source of proteins, amino acids and oils, A critical review. J. Microbiol. Biochem. Technol. 5, 107–129.

Inouye LS, Lotufo GR (2006). Comparison of macro-gravimetric and micro-colorimetric lipid determination methods. Talanta, 70, 584–587.

International Organization of Standardization (ISO) (1996). Animal and vegetable fats and oils-Determination of acid value and acidity (ISO 660:1996). Geneva, Switzerland: ISO Central Secretariat.

Jayed MH, Masjuki HH, Saidur R, Kalam MA, Jahirul MI (2009). Environmental aspects and challenges of oilseed produced biodiesel in Southeast Asia. Renewable and Sustainable Energy Reviews 13(9), 2452-2462.

Karkal SS, Kudre TG (2020). Valorization of fish discards for the sustainable production of renewable fuels. J. Cleaner Prod. 275, 122985.

Karkal SS, Kudre TG (2021). Optimization of biodiesel production from marine fish waste oil using calcined chicken eggshell as a cost-effective catalyst by response surface methodology. Available at SSRN: 10.2139/ssrn.3904620.

Karkal SS, Kudre TG (2023). Valorization of marine fish waste biomass and Gallus gallus eggshells as feedstock and catalyst for biodiesel production. Int. J. Environ. Sci. Technol. 20, 7993–8016.

Khan S, Naushad M, Iqbal J, Bathula C, Sharma G (2022). Production and harvesting of microalgae and an efficient operational approach to biofuel production for a sustainable environment. Fuel 311, 122543.

Knothe G (2010). Biodiesel and renewable diesel: A comparison. Progress in Energy and Combustion Science. 36(3), 364-373.

Kudre TG, Bhaskar N, Sakhare PZ (2017). Optimization and characterization of biodiesel production from rohu (Labeo rohita) processing waste.Renewable Energy. 113, 1408-1418

Ling JSJ, Tan TY, Mubarak NM, Kansedo J, Saptoro A, Nolasco-Hipolito A (2019). A review of heterogeneous calcium oxide based catalyst from waste for biodiesel synthesis. SN Appl. Sci. 1, 1–8.

Medeiros EF, Silva Afonso M, Santos MAZ, Bento FM, Quadro MS, Andreazza R (2019). Physicochemical characterization of oil extraction from fishing waste for biofuel production. Renew. Energy 143, 471–477.

Ong HC, Tiong YW, Goh BHH, Gan YY, Mofijur M, Fattah IR, Mahlia TMI (2021). Recent advances in biodiesel production from agricultural products and microalgae using ionic liquids: Opportunities and challenges. Energy Conversion and Management 228, 113647.

Piloto-Rodríguez R, Sanchez-Borroto Y, Lapuerta M, Goyos-Perez L, Verhelst S (2013). Prediction of the cetane number of biodiesel using artificial neural networks and multiple linear regression. Energ. Conver. Manage. 65, 255–261.

Tilamia K, Sabine Samples S, Sampels S (2018). Nutritional value of fish: Lipids, Proteins, Vitamins, and Minerals. Reviews in Fisheries Science & Aquaculture, 26(2), 2–5.

Wisniewski JrA, Wiggers VR, Simionatto EL, Meier HF, Barros AC, Madureira LAS (2010). Biofuels from waste fish oil pyrolysis: Chemical composition. Fuel 89(3), 563-568.