Available online at www.jpsscientificpublications.com
Volume – 1; Issue - 2; Year – 2015; Page: 115– 121

Indo – Asian Journal of Multidisciplinary Research (IAJMR)
ISSN: 2454-1370

PRODUCTION AND CHARACTERIZATION OF FATTY ACID METHYL ESTER FROM FISH OIL *Rastrelliger kanagurta*

G. Sathiya Rathna and M. Kalaiselvam*

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502, Tamil Nadu, India.

Abstract

The need for alternative energy sources that combine environmental friendliness with biodegradability, low toxicity, renewability and less dependence on petroleum products has never been greater. One such energy source is referred to as biodiesel but the resources are in demand one of the excellent alternative area is aquatic resources are an abundant, low-cost raw materials for production of biodiesel. About 25% of the total production of marine capture fisheries is discarded ever year which are having enough potential for fuel extraction. Fish oil rich in PUFA its improve fuel flow fluidity at low operating temperatures, in the same time high PUFA cause deterioration leads to precipitation of the biodiesel. In the present study discussed with raw fish oil can be used for biodiesel production, oil extracted from *R. kanagurta* by direct steaming method and phospholipids were removed by refining process. Very little amount of polyunsaturated fatty acid like DHA (0.25%) and EPA (0.29%) was recorded in GC-MS, which was suitable for biodiesel production. Biodiesel was produced from the extracted fish oil after the chemical reaction of transesterification resulted in almost complete conversion of fish oil to fatty acid methyl ester (FAME) was observed.

Key words: *R. kanagurta,* Fish oil, Transesterification and Biodiesel.

1.Introduction

Biodiesel, as mono-alkyl esters of fatty acids, is to be a clean alternative fuel that reduces pollutant emissions from combustion equipment such as unburned hydrocarbons (68%), carbon monoxide (44%), sulfur oxide (100%) and polycyclic aromatic hydrocarbons (90%) (Wu and Leung, 2011). It's suitable for diesel vehicles, coastal ships, and merchant vessels also results in fewer poisonous emissions into the atmosphere (Liaquat *et al*., 2010) Moreover, biodiesel that leaks or is discharged from marine vessels into the ocean generally decomposes into less harmful components and causes much less pollution to the ocean environment (Lin and Huang, 2012).

The basic materials of plant-derived oils, waste oils, fats and microbial oils are used for biodiesel production (Akoh *et al*., 2007). Plant-derived fats and oils are not ideal for use in biodiesel production because the crops can also be used as food, in the oleochemcial industries and as livestock feed (Jegannathan *et al*., 2008) Biodiesel industries would compete with chemical, food and livestock feed industries for plant sources (McNeff *et al*., 2008). An increased demand for these plants could increase fertilizer use, contributing to increased greenhouse gas emissions which are also a serious environmental concern. For example, there is a 70% increase in greenhouse gas emission due to biodiesel production from heavily fertilized plants. To find a low-cost, sustainable

Corresponding author*: **M. Kalaiselvam
Tel.: +91-9894445103
E-mail: kalaifms@gmail.com
Received: 07.03.2015; *Revised*: 29.03.2015;
Accepted: 05.04.2015.

supply of feedstock with adequate fuel properties is the premise driving the development of the biodiesel industry is marine based sources (Pinnarat and Savage, 2008). According to the Food and Agriculture Organization (FAO, 2006), the world fish production in 2006 was 141.6 million tonnes of fish, and around 50% of the total fish material processed becomes waste material. This represents 70.8 million tonnes of fish waste, of which the amount of oil varies from 40% to 65% (Aruda *et al*., 2007). Most of the wastes from the fish industries are disposed into the sanitary landfills and effluent treatment ponds are produces unpleasant odor in their nearby vicinity. Globally, 1/4 of the total fish catch is discarded, and these are either generated from fishery processing or as by-catch (Falch *et al*., 2006). Some of these discards used as sources of various valuable bio ingredients such as proteins, minerals and lipids. Fish oil is produced in large quantity by fish-processing industry (Sathivel *et al*., 2004).

Active studies are carried out for using fish oil as fuel for diesel engines (Steigers, 2000). Fish oil rich in polyunsaturated fatty acids mainly that include EPA and DHA this can be used as raw oil for biodiesel production the unsaturation improve fuel flow fluidity at low temperatures. These acids are produced by marine algae which are consumed first by zoo- plankton and then by fish. Biodiesel fuels produced from raw oil with longer chain fatty acids generally have a higher cetane number and thus superior compression ignition characteristics (Lin *et al*., 2006). The ignition quality of a diesel fuel is also affected by the degree of saturation of the fatty acid compounds, with a greater saturated fatty acid content resulting in superior ignition quality (Wadumesthrige *et al*., 2008).

Mackerel is a fatty species that belongs to Scombridae family, abundant in cold and temperate shelf areas; fat content is well-distributed throughout the body (Osman *et al*., 2001). Many species of marine fishes have been studied and subjected for fish oil production but little attention had been paid to the production of biodiesel from the same. Biodiesel production from fish may leads to the control of solid waste generated from fish industries and helps in improving Indian economy. Even though the use of fish oil as biodiesel has a great potential but research in this field is very limited. Hence, the present study was aimed to uncover the efficacy of mackerel fish to assess the advantages of biodiesel production.

Aim and Objectives

To revel that the marine *R. kanagurta* oil was used as alternative way for the production of biodiesel.

2. Materials and methods

Sample collection

Mackerel (*Rastrelliger kanagurta*) were collected from the marine landing, Annankovil (Lat. 11°29'N; Long. 79°46'E) of Parangipettai, South east coast of India.

Extraction of fish oil

Collected fishes were washed thoroughly in running water so as to remove sand and debris. Digestive systems were removed and remaining parts were subjected to extraction of oil by direct steaming method compromises of cooking, pressing, centrifuging was carried out and considered as the common method used in the extraction of fish oil. About 1000 g of homogenized fish tissue sample was taken in a muslin bag and kept in a steam boiler at 70-80^{0}C for 30 min. The boiled fish tissues were then pressed with the aid of fish oil extractor so as to remove the liquid content from the tissue (containing oil and water). Then the oil was separated from the water by centrifuging at 2000 rpm for 15 min and further by separating funnel. The filtered oil was stored separately in an opaque dark bottle and placed in deep freezer at 20^{0}C.

Crude oil purification steps

The oil obtained directly from rendering contain varying but relatively small amount of naturally occurring non-glyceride materials that were removed through series of processing steps.

Crude oil contains some free fatty acids, water, and protein was removed.

Degumming

One ml of phosphoric acid was added with 20 ml of distilled water and added to preheated 20 ml crude fish oil. The solution was heated in a mantle for 30 min at 50-60°C, after that they are placed in a separating funnel for some time for the separation of gums. Two separated layers appeared after few min, the lower layer was the lipid layer and the top layer was the oil layer. The lipid layer was disposed out using separating funnel. The oil was then mixed with equal amount of distilled water and thoroughly mixed and placed in the separating funnel for some time. The lipid was completely removed by washing with water.

Neutralization

The process of refining (sometimes referred to as "alkali refining") was performed on the degummed oil to reduce the free fatty acid content and to remove other impurities such as remaining phosphatides, proteinaceous and mucilaginous substances. Caustic neutralization was carried out using 16°C sodium hydroxide (lye solution) in 10% excess. The lye solution was added drop by drop to the degummed oil in an open beaker with constant vigorous stirring with magnetic stirrer at 70°C for 15 min, followed by 20 min settling time. The oil was then transferred to the separating funnel and washed to make it free from soap using 4 × 15% (v/v) hot deionised water washes, leads to a pH of 7 at the time of final water wash. Each wash was done at 100°C for 10 min followed by settling for 15 min. The soap water was then discarded. The oil was neither bleached nor deodorized. The oil was heated up to 150°C for 10 min on hot plate for the removal of moisture and odor to some extent.

Drying

To remove the water molecule present in the oil. 0.01g of calcium carbonate crystals were mixed with 1 ml distilled water and then mixed to the fish oil. The oil was heated to 95°C at atmospheric pressure. The moisture removed oil appeared in golden yellow color.

Deodorization

To deodorize the fish oil, charcoal was mixed with the water contained surf excel powder. The soaked charcoal was placed in open air for overnight shade drying. Then the charcoal was placed in the oil. Next morning this charcoal is put into the oil. After 3 hours, the oil was filtered out from the charcoal.

The fatty acid composition (%) of the saturated and unsaturated fatty acids of the fish oil was analyzed by GC-MS chromatography

Transesterification

Transesterification reaction was carried out using an alkali catalyst to produce the fish-oil biodiesel primarily owing to its dominant advantage of being applied widely in industrial mass production and the shorter reaction time required for biodiesel production. The molar ratio of methanol to the pre-treated fish oil was fixed at 6:1.1 wt.%. An alkali catalyst, NaOH was used to enhance the transesterification reaction. The reactant mixture was stirred in a reacting tank by a mechanical homogenizer at a speed of 6000 rpm for 50 min to complete the chemical reaction. The reaction temperature was set at 60°C, just below the boiling point of methanol at 63°C, to prevent the vaporization of methanol from the reactant mixture.

Separation process

After the completion of the transesterification reaction, the crude product was then separated into two layers, crude biodiesel and glycerol, because of the density difference through either keeping the product motionless or centrifuging it. The crude biodiesel was thereafter washed with distilled water several times until the washed water became clear.

3. Results and Discussion

In this study different size groups of *R. kanagurta* were examined for the extraction of oil and it was evident that 100ml of crude fish oil was

extracted from 1000 g of fish by the process of direct steaming method. The results clearly illustrates fatty fish groups are capable of producing a better yield of oil. Mackerel is the most favorite food fish and the success of its fishery determines the marine fishery scenario. Annual and seasonal fluctuations in catch are an inherent feature of the fishery. Multi-day trawl was introduced for further enhancement of mackerel catches. The difference in the size at first maturity observed in fish caught in different gears is mostly due to the effect of environment on the biology of the fish. The reduction in growth rate of mackerel when it moves to deeper waters (Yohannan, 1979). The trawls generally harvest mackerel from deeper waters and the smaller size at first fishery maturity observations (Rohit and Gupta, 2004).

Fish oil extracted from body tissues and fish wastes such as the viscera and skin. In the present study, totally 100ml of crude oil extracted from 1000 g body tissue of mackerel *R. kanangurtha*. The extracted crude fish oil contains phosphatides, gums and other complex compounds which can promote hydrolysis of oil during storage. Particularly the calcium and magnesium salt of phosphatidic and lysophatidic acids are strong emulsifiers. If these compounds remain in the oil during the later alkali neutralization step, they may inhibit the separation of the soaps and lowers the yield of neutral oil. Phospholipids will also react with water to form insoluble sediments. During transesterification process, these compounds can also interfere. Therefore, these compounds were removed by acid degumming process followed by Refining/Neutralization, Deodorization and Drying.

Totally 80 ml purified fish oil was obtained and GC-MS was performed to analyze the fatty acid profile. GC-MS was used to identify and measure the composition of various fatty acids present in fish oils (Table 1). The sequences of the fatty acids were ordered according to their chromatographic retention time. Although 64 fatty acids could be identified by morden technology, but only six pairs of fatty acid *viz*., C16:1; C18:0; C18:1; C20:1; C22:1; C22:6ω3 which cover 80 to 85 % are vital. The fatty acid content varied from 0.09 to 22.47%. In the present study, fatty-acid composition of marine fish oil biodiesel is more complex than that of general vegetable-oil biodiesels and consists of 5.66 wt.% oleic acid (C18:1), 10.11 wt.% palmitic acid (C16:0), 22.47 wt.% stearic acid (C18:0), and 22.47 wt.% Myristic acid (C14:0) 12. In addition, long chain polyunsaturated fatty acid (LC-PUFA) compounds such as docosahexaenoic acid (DHA, with a carbon chain structure C22:0) and eicosapentaenoic acid (EPA, with a structure C22:0) of marine fish-oil biodiesel are as 0.25 wt.% and 0.29 wt.%, respectively.

Usually mackerel fish species contain 0.4-1.85 of Omega 3 (EPA+DHA) content (g) per 100 g of fish (Tony Piccolo, 2011). Fish species and their lipid content were varied from the part like head (7.01%), intestine (4.46%) and liver (3.70%) of *E. affinis* lipid content was estimated (Khoddami *et al*., 2012). Extracted oil from the body tissue and head of *Monopterus albus* and the results examined lipid content 0.50 and 1.06 g/100 g wet tissue in the body and 0.40 and 0.78 g/100 g wet tissue in the head (Razak *et al*., 2001). Tanbirul Haque *et al*. (2014) obtained mackerel muscle oil by SC-CO_2 method and 4.00 ± 0.11 g/20 g of mackerel muscle at 45^{o}C with a pressure 25 MPa. The present results were in agreement with the data available on the fatty acid composition of fishes in previous report (Jamilah *et al*., 2008). Fish waste is opted as being a good source of lipid and it was evident in cod offal (4.30%) and sardine head (5.67%) (Khoddami *et al*., 2009).

The crude fish oil from mackerel *R. kanagurtha* contains a moderate amount of PUFA'S especially DHA (0.25%) and EPA (0.29%) which could be directly employed for biodiesel processing. The estimation of PUFA content is important because PUFA content is a part of the EN 14214 specifications of biodiesel. There is no existing standard method for the estimation of total PUFA content in biodiesel. The

developed GC-Ms method quantifies EPA and DHA (C20:5 and C22:6) fatty acid methyl esters which can be used as markers for the estimation of fish oil biodiesel. Very little amount of polyunsaturated compounds was observed. Highly presence of EPA and DHA indicates the contamination of fish oil in biodiesel (Chopra *et al*., 2011).

Table - 1: Fatty acid composition (%) of fish oil methyl ester

S.No	Carbon chain	Fatty acid	Content%
1	C10:0	Capric acid	0.44
2	C11:0	Undecyclic acid	0.06
3	C12:0	Lauric acid	0.11
4	C13:0	Tri decyclic acid	0.43
5	C14:0	Myristic acid	22.47
6	C15:0	Penta decyclic acid	0.09
7	C16:0	Palmitic acid	10.11
8	C17:0	Margaric acid	1.20
9	C18:0	Stearic acid	22.47
10	C18:1	Oleic acid	5.66
10	C19:0	Nonadecyclic acid	4.77
11	C20:0	Arachidic acid	4.72
12	**C22:0**	**Docosa hexaenoic acid**	**0.25**
13	**C22:0**	**Eicosapentaenoic acid**	**0.29**

Biodiesel with polyunsaturated fatty acids with more than three double bonds is prone to deterioration in its oxidation stability, thus causing the precipitation of the biodiesel components in a fuel feeding system or combustion chamber (Tanwar *et al*., 2013).

4. Conclusion

Thus, mackerel *R. kanagurtha* fish oil contains a little amount PUFA, which could be directly employed for biodiesel processing. Transesterification is the faster and an economical way for the conversion of fish oil to biodiesel.

Acknowledgements

The authors are thankful to the Dean, Faculty of Marine Sciences and the Authorities of Annamalai University for providing necessary facilities to carry out this work and also thank Ministry of Human Resource and Development (MHRD)**,** Government of India, for granting fellowships.

5. References

1) Wu, X and D.Y.C. Leung. 2011. Optimization of biodiesel production from C*amelina* oil using orthogonal experiment. *Appl. Ene.*, 88: 3615-24.

2) Liaquat, A.M., M.A., Kalam, H.H. Masjuki and M.H. Jayed. 2010. Potential emission reduction in road transport sector using biofuel in developing countries. *Atmos. Environ.*, 44: 3869-3877.

3) Lin, C.Y and T.H. Huang. 2012. Cost-benefit evaluation of using biodiesel as an alternative fuel for fishing boats in Taiwan. *Mar. Pol.*, 36: 103-107.

4) Akoh, C.C., S. Chang, G. Lee, and J. Shaw. 2007. Enzymatic approach to biodiesel production. *J. Agric. Food Chem.*, 55: 8995-9005.

5) Jegannathan, K.R., S. Abang, D. Poncelet, E.S. Chan and P. Ravindra. 2008. Production of biodiesel using immobilized lipase-a critical review. *Crit. Rev. Biotechnol.,* 28: 253-264.

6) McNeff, C.V., L.C. McNeff, B. Yan D.T. Nowlan and M. Rasmussen. 2008. A continuous catalytic system for biodiesel production. *Appl. Catal., A Gen.,* 343: 39-48.

7) Pinnarat, T and P.E. Savage. 2008. Assessment of non catalytic biodiesel synthesis using supercritical reaction conditions. *Industrial and Engineering Chemistry Research*, 47: 6801-6808.

8) Aruda, L.F., R. Borghesi and M. Oeterer. 2007. Use fish waste as silage-a review. *Braz. Arch. Biol. Technol.,* 50: 879-86.

9) Falch, E., M. Aursand and T. Rustad. 2006. By product from *Gadiform* species as raw materials for the production of marine lipids as ingredients in food or feed. *Process Biochem.,* 41: 66-674.

10) Hard, N.F., B.K. Simpson and Z.E. Sikorskiz. 1994. Biotechnological applications of seafood proteins and other nitrogenous compounds. In: Sikorski, Z.E., B. Sun Pan and F. Shahidi (Eds.) Seafood proteins. Chapman and Hal, New York, 194-216.

11) Sathivel, S., P. Bechtel, J. Babit, W. Prinyawiwatikul, I.I. Negulescu and K.D. Repond. 2004. Properties of protein powders from Arowtoth flounder (*Atheresthes stomias*) and hering (*Clupea harengus*) byproducts. *J. Agric. Fod. Chem.,* 52: 5040-5046.

12) Steigers, J.A., 2000. Demonstrating the use of fish oil as fuel in a large stationary diesel engine, In: advances in seafood byproducts 2002 conference proceedings. Bechtel, P.J., (Eds.) Alaska sea grant fair banks, AK. 187-200.

13) Blythe, N.X. 1996. Fish oil as an alternative fuel for internal combustion engines: American Society of Mechanical Engineers. Internal Combustion Engine Division (Publication) ICE. 26: 85-92.

14) Lin, C.Y., H.A. Lin and L.B. Hung. 2006. Fuel structure and properties of biodiesel produced by the peroxidation process. *Fuel,* 85: 298-305.

15) Wadumesthrige, K., A. Jeremiah, C. Smith, J.R. Wilson, A. Steven, O. Salley and K.Y.S. Ng. 2008. Investigation of the parameters affecting the cetane number of biodiesel. *Journal of American Oil Chemists Society,* 85**:** 1073-1081.

16) Osman, H., A.R. Suriah and E.C. Law. 2001. Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. *J. Food. Chem.,* 73: 55-60.

17) Yohannan, T.M., 1979. The growth patterns of Indian mackerel. *Ibid,* 26(1&2): 207-216.

18) Rohit, P., P.P. Pillai, A.C. Gupta and K. Preetha. 1998. Fishery and population characteristics of mackerel landed by trawlers along Dakshina Kannada coast. *Ibid,* 45(1): 21-28.

19) Rohit, P. and A.C. Gupta. Fishery, biology and stock of the Indian mackerel *Rastrelliger kanagurta* off Mangalore-Malpe in Karnataka, India. *J. Mar. Biol. Ass. India,* 46(2): 185-191.

20) Tony Piccolo, 2011. A feasibility study for fish oil bio-diesel production. Sustainable Communities Enterprise for Clayoquot Biosphere Trust. MBA. www.aquaticbiofuel.com.

21) Khoddami, A., A.A. Ariffin, J. Bakar and H.M. Ghazali. 2008. Quality and fatty acid profile of the oil extracted from fish waste (head, intestine and liver) (*Euthynnus affinis*). *African Journal of Biotechnology,* 11(7): 1683-1689.

22) Razak, Z.K.A., M. Basri, K. Dzulkefly, C.N.A. Razak and A.B. Salleh. 2001. Extraction and characterization of fish oil from *Monopterus Albus*. *Malaysian Journal of Analytical Sciences,* 7(1): 217-220.

23) Tanbirul Haque, A.S.M., A.K.M. Asaduzzaman and Byung-Soo Chun. 2014. Fatty acid composition and stability of extracted mackerel muscle oil and oil-polyethylene glycol particles formed by gas saturated solution process. *Fish Aquat. Sci.,* 17(1): 67-73.

24) Jamilah, B., R.E. Zakipoor and Y.B. Che. 2008. Man Lipid characteristic in cooked, chill-reheated fillet of Indo-Pacific king mackerel (*Scomberomorous guttatus*), LWT- Food. *Sci. Technol.,* 41(10): 2144-2150.

25) Shahidi, F., M. Naczk, R.B. Pegg and J. Synowiecki. 1991. Chemical composition and nutritional value of processing discards of cod (*Gadus morhua*). *Food Chem.*, 42: 145-151.

26) Khoddami, A., A.A. Ariffin, J.Bakar and H.M. Ghazali. 2009. Fatty acid profile of the oil extracted from fish waste (Head, Intestine and Liver) (*Sardinella lemuru*). *World Applied Sciences Journal,* 7(1): 127-131.

27) Chopra, A., A.K. Tewari, S. Vatsala R. Kumar, A.S. Sarpal and B. Basu. 2011. Determination of polyunsaturated fatty esters (PUFA) in biodiesel by GC/GC–MS and ^{1}H-NMR Techniques. *Journal of Oil and Fat Industries,* 88(9): 1285-1296.

28) Tanwar, D., Ajayta, D. Sharma, Y.P. Mathur, K. Kishore, Khatri, S.L. Soni, R. Gupta. 2013. Production and Characterization of Fish Oil Methyl Ester. *International Journal of Innovative Technology and Research*, 1(3): 209-217.