Isolation of Omega 3 Fatty Acid from Fish Oil

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ABSTRACT

Omega 3 fatty acids containing Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) play a beneficial role in human health and as functional food. In this study fish oil was purchased from Fish oil and Fish meal extraction factory, Mangalore. The standards were purchased from sigma Aldrich. The fish oil was further characterized by various analytical techniques such as High performance Liquid chromatography (HPLC), thin layer chromatography (TLC), Fourier infrared spectroscopy (FTIR) in order to isolate omega 3 fatty acids from fish oil. Quality of fish oil was analysed in order to determine impurities, acid value and peroxide value of fish oil which was in recommended range. Traditional extraction of omega 3 fatty acids are expensive and time consuming. Basically high yield and concentrated omega 3 fatty acids from sardine fish oil were obtained by supercritical fluid extraction, urea complexation, low temperature crystallization and molecular distillation, enzymatic hydrolysis. The main aim to enhance and improve the concentration of polyunsaturated omega 3 fatty acids from saradine fish oil and production of biodiesel through Tansesterification after recovery of omega 3 fatty acids.

Keywords: ***Omega 3 Fatty acid, Enzymatic Hydrolysis, Transesterification***

1. INTRODUCTION

Fish is one of the perishable human food overall the world. Its nutritional value includes phosphorous, magnesium, selenium. Fish oil which is rich in EPA and DHA helps in the reduction of heart attack, strokes, abnormal heart rhythms, death [1]. Omega 3 are the polyunsaturated fatty acids having carbon carbon as double bond in its position [2]. Determination of omega lipids in the fish oil comprises of several steps including extraction, hydrolysis and derivatization and making UV active compound for measured by HPLC [3].

HPLC with UV detector was a challenging task by making sample derivatization helped to isolate an omega fatty acids in fish oil [4]. EPA and DHA from fish oil content varies from species to species and season. Conversion of fatty acids to ethyl esters followed by molecular distillation, urea crystallization damages the omega 3 fatty acids. Re-esterification of fatty acids leads to a disturbance in backbone of glycerol [5].

To overcome this destruction and challenges, lipase is used for concentrating omega fatty acids due to its important properties [6-7].

2. MATERIALS AND METHODOLOGY

2.1 Determination of acid value:

The acid value of the sardine fish oil sample was determined according to the standard procedure described in the American Oil Chemists Society (AOCS). 25 mL of 1:1 toluene-isopropyl alcohol and 2 mL of phenolphthalein indicator were added into 250 mL conical flask and neutralized with 0.1N potassium hydroxide to a faint but permanent pink color. In another conical flask, 0.75 g of oil sample was weighed and 25 mL of neutralized solvent mixture were added to the sample and mixed thoroughly. Titration was then carried out with 0.1N potassium hydroxide to permanent pink color. The acid value (mg KOH/g of sample) was given by the following formula [8].

$$\text{Acid value} = \frac{(A-B) \times N \times 56.1}{W} \quad (1)$$

A= mL of standard alkali used in the titrating the sample
B= mL of standard alkali used in the titrating the blank
N= normality of standard alkali
W= grams of sample
56.1 is molecular weight of KOH in grams.

2.2 Determination of p-Anisidine value

The *p*-Anisidine value of sardine fish oil sample was determined using the standard protocol described in the AOCS official method (AOCS). 0.7 g of oil was added into a 25 mL volumetric flask.Volume was made up with diluted iso-octane. The absorbance was measured at 350 nm with spectrophotometer. 5:1 ratio of iso-octane and of *p*-anisidine reagent were used as blank. 5:1 ratio of sardine oil and p anisidine reagent was added to test tube and taken for UV analysis after 10 minutes; the absorbance was measured at 350 nm. The *p*-anisidine value was given by the following formula[8-9].

$$p\text{-anisidine value} = \frac{25 \times (1.2\ A_S - A_B)}{W} \qquad (2)$$

Where:

A_S = absorbance of the oil solution: *p*-anisidine reagent

A_B= absorbance of the oil solution

W= weight of sample

25= size of volumetric flask used

1.2= correction factor

2.3 Chemical hydrolysis of fish oil

The fatty acid of sardine fish oil composition has been previously stated[9].Among the fatty acids such as EPA (15.6%) and DHA (10.7%), the hydrolysis of sardine fish oil was performed in an organic and aqueous system. The protocol was as follows: 2mL fish oil was added into 20mL of distilled water along with 2g of NaOH and refluxed for around 8h at 100^0C.

2.4 Thin layer chromatography

The various fatty acids are separated and fractionated by thin layer chromatography. The lipids were fractioned using hexane/ethyl acetate (75:25). The conditions for analysis of fish oil were established by experimental selection of appropriate stationary and mobile phases. Experiments were performed on TLC silica gel aluminum plate. Good separations were obtained in short time using mobile phase Ethyl acetate (25%): Hexane (75%). TLC plate was spotted with crude sample and esterified sample. Plates were then air dried and analysed in UV chamber [4].

2.5 Preparation of UV absorbing derivatives

0.1-0.2 g of fish oil was transferred into round bottom flask. It was dissolved in 2mL of acetonitrile and around 0.4 g of 4-Nitrobenzyl bromide is added and exactly 0.2 g of potassium carbonate was added in RB flask. The reaction mixture was kept stirring at room temperature for 18 h. After refluxing the sample was allowed to settle down and around 50 microliter of sample was taken for analysis of TLC to check the state of reaction. Remaining refluxed mixture is subjected to phase separation using separating funnel and extracted with ethyl acetate. Aqueous phase was separated and discarded and further organic phase was concentrated using rotary evaporator and subjected to HPLC analysis.

2.6 Characterization of fish oil by FTIR Spectroscopy

FTIR spectra of sardine fish oil were analyzed on a Perkin Elmer RXI. The FTIR spectra were acquired from 3850-400 cm^{-1} with 4 cm^{-1} resolution by 20 scans with, monochromatic infrared radiation as source, with LiTa 03 detector.

2.7 Concentration of n-3 fatty acids by enzymatic hydrolysis

4g sardine fish oil, 6 mL of 1M phosphate buffer to activate lipase enzyme and maintained pH of 7.5 and 1,150 Units (600 U/g) of lipase were transferred into a 50 mL conical flask. The flask was transferred to water bath by maintaining temperature at 35°C. The hydrolysis was started with the constant stirring for 18 hours. The hydrolysis process was stopped by addition of 2 mL methanol to the mixture. An amount of base was added to neutralize the fatty acids obtained during hydrolysis. The mixture was taken into a separating funnel and mixed thoroughly with 50 mL hexane and 25 mL distilled water. The upper layer containing ethyl esters was separated and was washed twice with 50 mL distilled water. In order to remove moisture content it is subjected with anhydrous sodium sulphate [10] .After hexane removal at 45°C, ethyl esters were recovered in a rotary evaporator.

2.8 Transesterification reaction

Transesterification was carried out in 50mL conical flask with 14mL of pure methanol and kept flask on the stirring plate with constant speed to stir vigorously, and then slowly 0.50g of NaOH added. Once NaOH is dissolved completely it forms methoxide of sodium which serves as very strong and dangerous base. Now place 30mL of fish oil which is warmed at 65°C by placing it on stir plate under medium agitation, then later add slowly the sodium methoxide solution to it which forms a cloudy appearance and reaction mixture was kept for around 20 minutes. Then transfer the above mixture into separating funnel were biodiesel and glycerol was separated based on their density [11].

3. RESULTS AND DISCUSSIONS

3.1 Quality of fish oil

Quality of fish oil was determined by standard AOAC method. The obtained anisidine and acid value were in recommended range.

3.2 FT-IR Spectrum

FTIR spectra of fish oil is shown in the figure 1, group frequency ranging from 1750-1725 cm^{-1} clearly indicates esters functional group ,by seeing figure 6 which clearly indicates the presence of esters in the range 1745.84 cm^{-1}.

3.3 EPA Standard

Figure 2, shows a typical Chromatogram of Eicosapentaenoic Acid (EPA), a single fatty acid was found to have 92.63 area percent of fatty acid DHA Standard.

Figure 3 shows a typical Chromatogram of Docosahexaenoic Acid (DHA), a single fatty acid was found to have 85.73 area percent of fatty acids.

3.4 Chemical method of separation

A chromatogram for chemically hydrolyzed fish oil is shown in the figure 4. Here a single fatty acid was found to have 0.4 area percent of the omega 3 fatty acids.

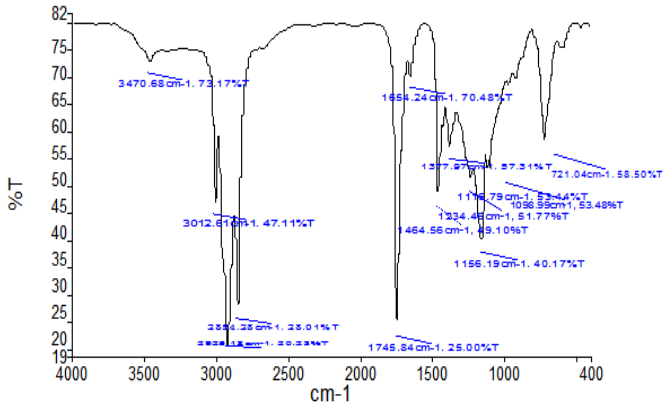

Figure 1. FTIR spectra (4000-400cm-1) of fish oil

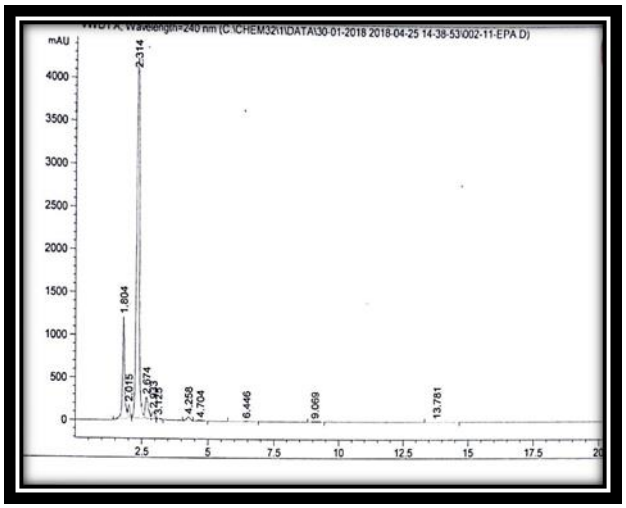

Figure 2. Chromatogram of EPA standard

Table 1: Recommended quality parameters of fish oil and Experimental values

Quality Parameter	Recommended Value	Experimental value	References
Acid Value	7-8 mg of KOH/g of sample	7.48mg of KOH/g of sample	12
p-anisidine value	≤20	19.575	13

3.5 Enzymatic method of separation

A chromatogram for enzymatic hydrolyzed fish oil is shown in the figure 5. Here a single fatty acid was found to have 2.0826 area percent of the omega 3 fatty acids.

3.6 Biodiesel yield and its properties

Transesterification of fish oil resulted in the reduced kinematic viscosity and density was within permissible limit which plays an important role in fuel atomization. The flash point, fire point were in limit of safe storage and handling conditions.

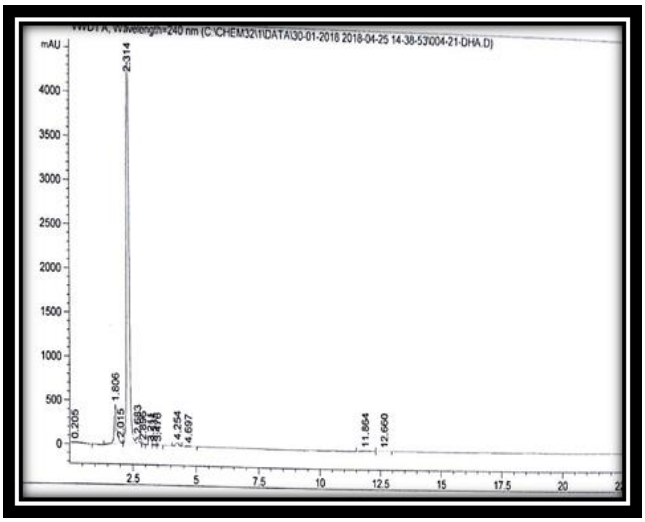

Figure 3. Chromatogram of DHA standard

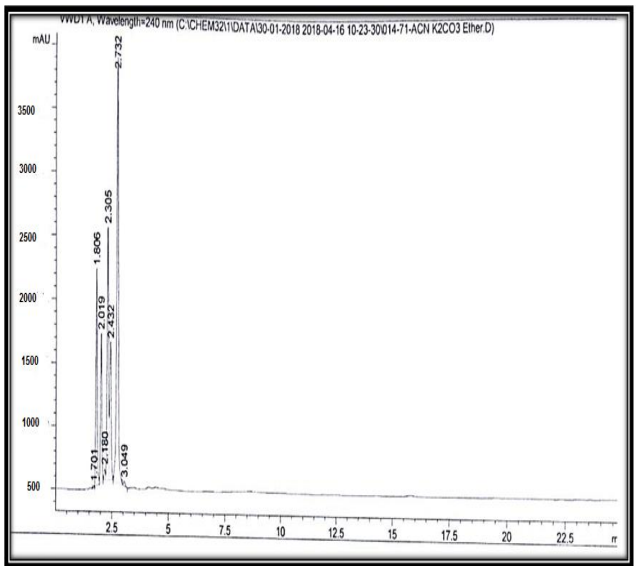

Figure 4. Chromatogram of chemical method of separation by HPLC

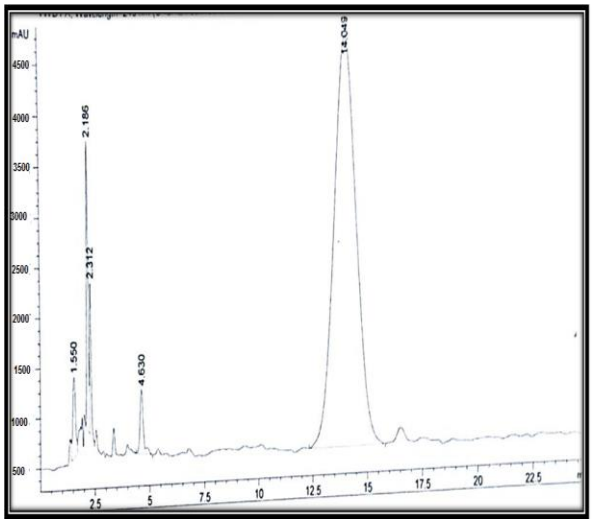

Figure 5. Chromatogram of enzymatic method of separation by HPLC

Table 2: Fuel properties of sardine fish oil ethyl esters

Parameters	Obtained value	Expected value
viscosity at 40°C	3.41	20
Flash point(°C)	170	200
Fire point(°C)	180	215
Density kg/m^3	830	920

4. CONCLUSION

Quality of fish oil was carried out by standard AOAC method. The recommended range for p anisdine value of crude fish oil is 7-8 mg of KOH /g. The obtained p anisidine value were in the range of 7.48 mg of KOH /g of the sample. Anisidine value is an empirical test which determines the advanced oxidative rancidity of oils and fats. The obtained acid values were in the recommended range, the acid values quantifies the amount of acid present, TLC analysis helped in quantitative identification of fatty acids present in fish oil. FTIR spectroscopy of the sample clearly indicated that frequency ranging from 1745.84 cm^{-1} clearly indicated esters functional group.

Using various Analytical techniques, it is possible to isolate different omega free fatty acids. Samples were hydrolyzed to separate the fatty acids from their glycerol backbone and analyzed directly using HPLC with UV detector. Chemical method of separation shown that 0.4 area percent of omega 3 fatty acids were as enzymatic method of separation 2.0826 area percent of omega 3 fatty acids. Transesterification of fish oil resulted in reduced kinematic viscosity and density within permissible limit which meets and well within the biodiesel characteristics as compared.

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