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Comprehensive Spectroscopic and Chromatographic Analysis of Waste Fish Oil Biodiesel using NMR, GC-MS, and FTIR Techniques for Sustainable Alternative Fuel Production

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This study presents a comprehensive characterization of wastes fish oil biodiesel using spectroscopic and chromatographic techniques, including Gas Chromatography-Mass Spectrometry (GC-MS), Fourier Transform Infrared Spectroscopy (FTIR), and Nuclear Magnetic Resonance (NMR) spectroscopy. The primary objective was to evaluate the molecular composition, purity, and structural integrity of biodiesel derived from waste fish oil through transesterification. GC-MS analysis identified a diverse range of fatty acid methyl esters (FAMEs), including saturated, monounsaturated, and polyunsaturated esters, reflecting the high degree of unsaturation characteristic of waste fish oil. The analytical results show successful transesterification of the detection of methyl tetradecanoate, 9-hexadecenoic acid methyl ester, and polyunsaturated eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) FAMEs. The characteristic ester carbonyl (1745 cm^{-1}), aliphatic chain (2923 cm^{-1} and 2853 cm^{-1}) and double bond (1650–1600 cm^{-1}) absorption bands detected through FTIR Spectroscopy confirmed the investigation conclusions. The NMR spectroscopy provided molecular information through ^{1}H-NMR and ^{13}C-NMR spectra which demonstrated that methyl esters appeared with signals at δ 3.6–3.7 ppm and δ 51.0–51.8 ppm while unsaturation levels became evident in the δ 127.0–130.0 ppm region. The results emphasis the process optimization because analysis shows the presence of phthalate derivatives and residual glycerol derivatives as well as other minor impurities. This study highlights the potential of waste fish oil as a sustainable feedstock for biodiesel production, contributing to renewable energy solutions and addressing environmental challenges.

Keywords: Waste fish Oil; biodiesel; transesterification; GC-MS Analysis; FTIR Spectroscopy; NMR Spectroscopy

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Growing international demand for sustainable power resources continues to escalate because of environmental protection goals combined with the necessary reduction of greenhouse gases and fossil fuel dependence. Biodiesel proves itself as an attractive replacement for petroleum-based diesel through its ability to biodegrade naturally while also being harmless to living systems and its effectiveness for cutting down greenhouse gas emissions. Biodiesel is made through the transesterification reaction which transforms triglycerides obtained from vegetable oils along with animal fats along with waste oils into Fatty Acid Methyl Esters (FAMEs). This production process originates from natural feedstock materials. FAMEs possess similar fuel characteristics to petro-diesel and additionally enhance lubrication properties and decrease particulate matter emissions as reported [1, 2]

Fish oil, a byproduct of the fish processing industry, represents an underutilised yet highly viable feedstock for biodiesel production. Rich in Poly Unsaturated Fatty Acids (PUFAs) such as Eicos-Apentaenoic Acid (EPA) and Docosa-Hexaenoic Acid (DHA), fish oil not only provides a unique composition of FAMEs but also contributes to favourable cold-flow properties and enhanced oxidative stability when stabilized with additives. However, the high degree of unsaturation in fish oil-derived biodiesel necessitates rigorous analytical characterization to ensure compliance with international fuel standards, such as ASTM D6751 and EN 14214 [3, 4].

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Spectroscopic and chromatographic techniques play a pivotal role in the comprehensive analysis of biodiesel. Gas Chromatography-Mass Spectrometry (GC-MS) provides the identification and summary of each FAMEs, providing overview into the biodiesel's molecular composition and relative availability of saturated, monounsaturated, and polyunsaturated esters. Fourier Transform Infrared Spectroscopy (FTIR) complements GC-MS by elucidating functional groups and confirming the presence of ester carbonyl groups, aliphatic chains, and unsaturated bonds. Nuclear Magnetic Resonance (NMR) spectroscopy, particularly ^{1}H-NMR and ^{13}C-NMR, offers detailed molecular fingerprints that validate the transesterification process and assess the purity and structural integrity of the biodiesel [5, 6].

The existing research studies have demonstrated the feasibility of producing biodiesel from various feedstocks, including sunflower oil, rocket seed oil, and jojoba oil, using optimized transesterification processes. In another research work investigation on the production and characterization of biodiesel derived from sunflower oil via alkali-catalyzed transesterification. They optimized reaction conditions (6:1 methanol-to-oil molar ratio, 0.7% NaOH catalyst, 60°C, 1.5 h) to achieve an 85.1% practical yield, validated by ^{1}H NMR analysis concluding 87.33% triglyceride conversion. The synthesized biodiesel exhibited fuel properties—including kinematic viscosity (4.719 mm²/s), density (0.86 g/cm³), flash point (183°C), and acid number (0.07 mg KOH/g)—that complied with ASTM D6751 standards. Spectroscopic (FT-IR, NMR) and chromatographic (GC-MS) analyses confirmed the formation of 11 fatty acid methyl esters (FAMEs), comprising saturated, monounsaturated, and diunsaturated esters. The research showed that sunflower oil represents a suitable raw material for commercial biodiesel manufacturing because it meets all required quality standards at high conversion rates. It also demonstrates sunflower oil's position as a sustainable biodiesel option, which supports worldwide efforts to implement renewable energy systems [7, 8].

The study on advanced NMR spectroscopic methods for biodiesel chemical analysis. According to the research analysis biodiesel molecular composition was examined through combined use of ^{1}H NMR and ^{13}C NMR spectroscopy which was supported by two-dimensional NMR methods HSQC and HMBC. The collected ^{1}H NMR spectra demonstrated effectiveness in measuring unsaturated groups through specific identification of signals for isolated and conjugated along with non-conjugated double bonds as well as carbonyl and other structural components. The researchers used the peak area integration at 5.31 ppm to measure double bonds while calculating 146.7 double bonds in each 100-molecule biodiesel sample. The quantification of methyl esters through the 3.63 ppm peak showed 98.7% transesterification efficiency. This study proved that NMR spectroscopy operates efficiently as an analytical technique for biodiesel quantification to monitor its chemical attributes both in production and storage environments [9, 10].

The comprehensive analysis was presented in "Identification, FT-IR, NMR (^{1}H and ^{13}C) and GC/MS studies of fatty acid methyl esters in biodiesel from rocket seed oil" regarding the successful base-catalyzed transesterification synthesis of biodiesel from rocket seed oil. Their spectroscopic tests revealed two major absorption bands in the FT-IR spectrum which established that esters had formed at 1742 cm^{-1} for carbonyl groups and 1168 cm^{-1} for C-O stretching. A distinct peak at 3.65 ppm in 1H NMR spectroscopy corresponded to methoxy protons of esters where transesterification reached 88.49% efficiency in triglyceride conversion into methyl esters. Signals at 174.26 ppm for the carbonyl carbon and other peaks showing lipid chain appeared in the ^{13}C NMR spectrum. The GC/MS testing of the biodiesel product detected eleven fatty acid methyl esters from C16 to C24 which proved the biodiesel's chemical complexity. The successful biodiesel synthesis was confirmed through these results which also provided details about its molecular structure thus demonstrating rocket seed oil's potential as a sustainable fuel source [11, 12].

An extensive investigation of different catalysts for jojoba oil transformation into biodiesel while emphasizing organotin compounds was conducted. The confirmation of biodiesel conversion through FT-IR spectroscopy showed specific ester absorption bands, which indicated the formation of esters compared to the original oil spectrum. The NMR spectroscopy revealed detailed information about molecular structure alterations that occur after the transesterification process. A peak at 3.66 ppm in the ^{1}H NMR spectrum of biodiesel showed a single strong signal from methoxy protons, which confirms the presence of biodiesel methyl esters. The disappearance of glyceridic methylene and methine protons signals confirmed the biodiesel production based on their typical presence in oil spectra. Additional confirmation about biodiesel synthesis success came from ^{13}C NMR spectroscopy, which showed both characteristic methyl ester signals and the removal of specific signals that were present in the oil. Biodiesel composition analysis was performed using GC-MS, which verified the conversion process by identifying all present fatty acid methyl esters in the produced biodiesel. Spectroscopic analysis played a crucial role in validating both chemical reactions and confirming the standard-compliant characteristics of biodiesel produced from jojoba oil [13, 14, 15].

This study distinctly incorporates spectroscopic (FTIR, NMR) and chromatographic (GC-MS) methods into the molecular-level characterisation of waste fish oil biodiesel. While previous research focused on analytical techniques, this study's integrated approach reveals the structural integrity, purity, and composition of functional groups in waste fish oil biodiesel. Increasing focus in alternative feedstocks for fossil

fuels needs to explore the detailed molecular characterization of waste fish oil biodiesel, especially its unsaturated fatty acid methyl esters (FAMEs) and potential impurities for sustainable fuel. This study attempts to address this gap by determining and quantifying important FAMEs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), while identifying minor impurities that could hinder storage stability [16, 17, 18].

MATERIALS AND METHODOLOGY

This research work employed waste fish oil as the feedstock for biodiesel production by the transesterification process. The transesterification was performed using methanol as a solvent and NaOH as the catalyst. The ratio of methanol to oil, the temperature and time of the reaction were optimized to maximise the conversion efficiency. The biodiesel produced was characterized using spectroscopic and chromatographic techniques.

Sample Preparation

All experimental samples were prepared in line with laboratory standards to achieve homogeneity and reproducibility. The liquid samples were dissolved in deuterated chloroform ($CDCl_3$) which has tetramethylsilane (TMS) as the internal standard. For FTIR analysis, the liquid samples were placed in quartz cuvettes while the solid samples were prepared as aluminium discs. Following the standard sample preparation, contamination was minimized to maintain precision in the results. Also, the formation of air bubbles has been avoided to ensure the homogeneity.

Gas Chromatography Mass Spectroscopy Methodology

The GC-MS analysis was performed using an Agilent 8890 GC system coupled with an Agilent 5977 MSD mass spectrometer, following a standardized acquisition method. The GC system was equipped with a capillary column (30 m × 250 µm × 0.25 µm film thickness) coated with HP-5ms stationary phase, suitable for a temperature range of -60 °C to 325 °C (maximum operational limit: 350 °C). The oven temperature program began at an initial temperature of 75 °C with a hold time of 0.5 minutes, followed by a ramp rate of 5 °C/min to 180 °C (held for 3 minutes), and then another ramp at 5 °C/min to 300 °C (held for 5 minutes). The total run time was 53.5 minutes, including a post-run cooling period of 1 minute at 50 °C. Helium was used as the carrier gas in constant flow mode, with a flow rate of 1.2 mL/min at the inlet, corresponding to an average linear velocity of 40.402 cm/sec. The injector was operated in split mode with a split ratio of 15:1, and the total flow rate was set to 22.2 mL/min, while the septum purge flow was maintained at 3 mL/min. The injector temperature was maintained at 250 °C to ensure complete vaporization of analytes. A sample volume of 1 µL was injected using an autosampler, with pre-injection and post-injection solvent washes performed using solvent A (4 washes each) at a volume of 8 µL. Sample washes were conducted twice with a draw speed of 300 µL/min and a dispense speed of 3000 µL/min.

The mass spectrometer was operated in Electron Ionization (EI) mode with a fixed electron energy of 70 eV. The ion source temperature was maintained at 230 °C, while the quadrupole temperature was set to 150 °C. Mass spectra were acquired in scan mode, covering a mass range of 50–600 m/z with a scan speed of 1,562 scans per second. A solvent delay of 3 minutes was applied to avoid detector saturation from volatile solvents. The transfer line connecting the GC to the MS was maintained at 280 °C to prevent analyte condensation. The TIC served for qualitative and quantitative analysis purposes, while BPC remained disabled in the system. The Laboratory processing software allowed precise target detection and measurement by analyzing mass spectral profiles. The specification of GC-MS instrument used for analysis is given in table 1.

Table 1. Specification of GC-MS instrument.

GC oven temperature	Near ambient to 450 °C
Chromatographic performance	Area repeatability: <0.5% Retention time repeatability <0.008%
Inlet split ratio	7500:1
Mass filter	Heated monolithic hyperbolic quadrupole
Ionization modes	Electron Impact (EI) and Chemical Ionization (CI)
Mass range	(m/z) 1.6 −1,050 amu
Mass resolution	Unit mass
Mass accuracy	1 µL injection of 100 a pg/µL OFN standard scanning from 50.350 u will give its monoisotope at m/z 271 ±0.005
Spectral accuracy	1 µL injection of a 100 pg/µL OFN standard scanning from 50.350 u will give 99.0% spectral accuracy
Ion source temperature	150-350 °C
Quadrupole temperature	106-200 °C
Detector triple	Axis detector with high energy dynode and long-life electron multiplier

Fourier Transform Infrared Spectroscopy Methodology

The FTIR spectroscopic testing was carried out with BRUKER RFS 27 MultiRAM FT-Raman Spectrometer that uses the Raman effect through inelastic light scattering principles. The analytical technique determines wavelength changes together with light intensity modifications from molecular vibrations and crystal lattice effects to identify detailed characteristics of sample compositions and structures. Between 4000 and 50 cm^{-1} the instrument measures spectrum range but uses the Nd:YAG laser device working at 1064 nm wavelength to both activate efficiently and reduce fluorescence interference. The ultra-high sensitivity equipped detector operates through LN_2-cooled germanium technology maintaining stable performance for five consecutive days without requiring additional cooling systems.

The spectrometer offered a selection of resolutions between 0.8 cm^{-1} and 4 cm^{-1} and had wavenumber accuracy calibrated to ±0.1 cm^{-1} for ensuring measurement accuracy and repeatability. The sample preparation method varied according to physical state: aluminum discs received 50 mg of solid material whereas 2 mL of liquid sample was placed into a quartz cuvette to prevent air bubbles and ensure uniformity. All experimental samples requiring optical clarity were settled on either quartz cuvettes or aluminum discs according to their physical condition. The instrument contained all necessary called ^{19}F allowing observation along with amplifier setting of 300 W on low frequency spectrum and 100 W on high frequency spectrum and applying high-band and broadband pre-amplifiers.

Standards for data collection included measurements spanning the entire spectral region from 4000 to 50 cm^{-1} with ±0.1 cm^{-1} wavenumber precision and required background correction for eliminating CO_2 and water vapor interferences. The spectrum quality improved through baseline correction protocols. The BRUKER proprietary software processed the acquired spectra to identify peaks through their characteristic wavenumber and to conduct quantitative analysis of peak intensities relative to one another. The system operated with advanced quality control procedures that included repeated reference material calibration checks and sample testing three times and regular detector sensitivity and laser stability checks. The applied methodology delivered reliable molecular vibration characterization with structural feature analysis which led to essential chemical composition insights of the samples. The specification of FTIR spectrometer used for analysis is given in table 2.

Nuclear Magnetic Resonance Spectroscopy Methodology

Nuclear Magnetic Resonance (NMR) spectroscopy operated as the main technique for molecular systems structure analysis. The solution-state NMR analysis of multi-nuclei systems operated through an AVANCE III 500 MHz NMR spectrometer. The instrument contains a superconducting magnet which reaches 11.7 Tesla while supplying 500 MHz proton resonant frequency. The actively shielded magnet provides 5.4 cm standard bore size which contains both built-in cryo-shims and 34-channel room temperature shims for achieving maximum field homogeneity.

The spectrometer is equipped with four RF channels, enabling triple-resonance experiments with a phase resolution of > 0.1 degrees and a frequency resolution of > 0.1 Hz. Amplifier power settings include 300 W (low frequency) and 100 W (high frequency), along with high-band and broadband pre-amplifiers. The shaped pulse generation is supported across all RF channels, and dual 21-bit analog-to-digital converters (ADCs) are used for enhanced signal acquisition. The data acquisition and processing were performed using TOPSPIN 2.1 software. Specialized probes with distinct setup capabilities handled the widest possible spectrum of experimental nuclei requirements.

Table 2. Specification of FTIR spectrometer.

Spectral range	4000-50 cm^{-1}
Source	Nd-YAG laser 1064nm
Detector	LN2 cooled ultra-high sensitivity Ge detector, 5 days hold time
Resolution	0.8 to 4 cm^{-1}
Wavenumber accuracy	0.1 cm^{-1}
Sample holders	Quartz cuvette and Aluminium disc
Sample required	50 mg solid (or) 2ml liquid
Types of samples	Solid, liquid, thin films and semisolid

1. The probe (BBO) allows observation of nuclei between 109Ag and 31P with 1H decoupling capability including automated 2H lock and tuning/matching procedures. The variable temperature (VT) mode allows this device to function from -150°C to +180°C.

2. The 5 mm Quadrupole Inverse Probe with Gradient (QXI) allows inverse detection of ^{1}H while performing simultaneous ^{15}N ^{13}C ^{19}F decoupling in addition to ^{2}H lock stability features and variable temperature operation from -150°C to +180°C.

Samples were processed through solution of liquids in deuterated chloroform ($CDCl_3$) that contained tetramethyl silane (TMS) as internal standard at different volume levels between 0.5 and 0.7 mL. The appropriate deuterated solvents were used to dissolve or suspend solid samples to achieve homogeneity and avoid aggregation.

The testing parameters remained constant for both ^{1}H-NMR plus ^{13}C-NMR spectroscopic methods. The analytical approach included adequate spectral width modifications to reach the mentioned chemical shift areas together with acquisition time optimizations for optimized resolution combined with run duration and specific relaxation periods for nuclei relaxation between scanning points. The method of proton decoupling was integrated into the ^{13}C-NMR experiments to generate simplified spectra by creating singlets from multiplet structures. The specification of NMR spectrometer used for analysis is given in table 3.

Table 3. Specification of NMR spectrometer.

Frequency	500 MHz
Magnetic Field	11.7 Tesla
Major Specifications / Accessories available	a) 5 mm BBO probe with gradient facility and auto sampler with VT facility. Tuning ranges from ^{109}Ag to ^{31}P also observation of 19F with 1H decoupling b) 3,2 mm CP/MAS probe with VT facility. Tuning ranges from ^{15}N to ^{31}P $+^{1}H+^{19}H$
Type of measurements / Analysis available	1D NMR, 2D NMR, Multi-nuclear NMR; Variable temperature measurements.
Sample Requirement	For Liquid Sample: 5 mg for ^{1}H NMR and 50 mg for ^{13}C NMR experiment. Compounds should be highly pure and soluble in commonly available solvents. Solubility, nature of compound [carcinogenic, toxic, lachrymatory, explosive, hygroscopic] and Structural formula [contemplated / known] to be mentioned. The sample must be soluble in 0.6 ml of deuterated solvent. Facilities available for 1D NMR, 2D NMR, Multi-nuclear NMR; Variable temperature measurements. RADIOACTIVE MATERIAL should not be submitted.

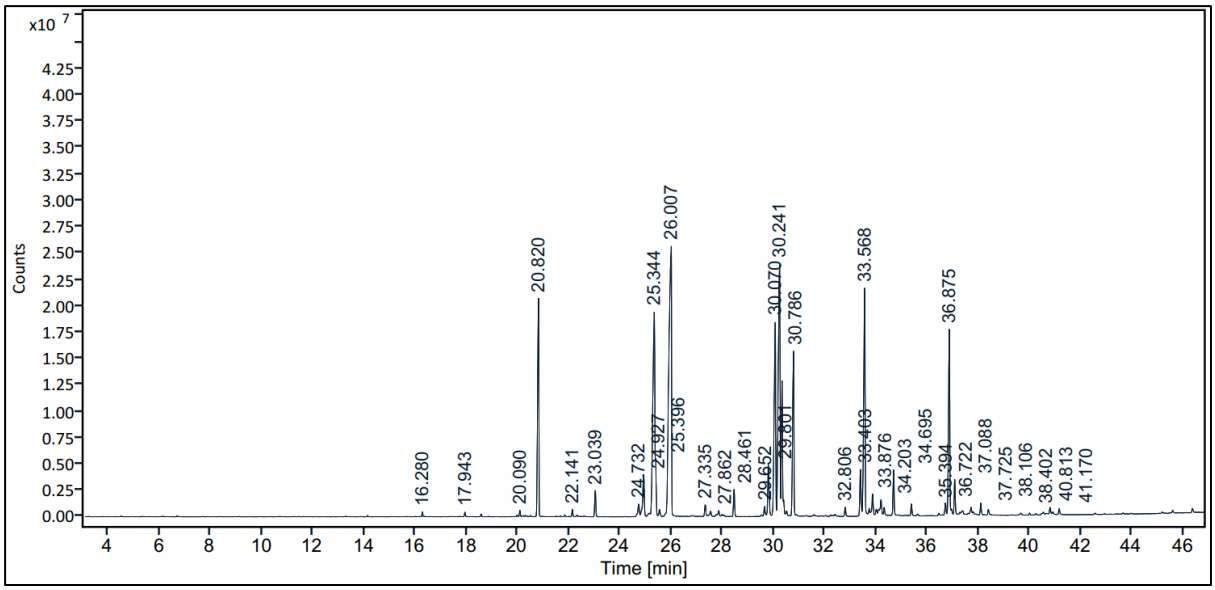

Figure 1. Total ion chromatogram of Gas Chromatography and Mass Spectroscopy of waste fish oil biodiesel.

RESULTS AND DISCUSSION

Spectrum Analysis of Gas Chromatography Mass Spectroscopy

The total ion chromatogram of gas chromatography and mass spectroscopy of waste fish oil biodiesel is shown in figure 1 and table 4 consolidated the list of major compounds identified using gas chromatography and mass spectroscopy. The Waste fish oil triglycerides underwent transesterification to produce a complicated FAME mixture which was analysed by GC-MS. The various FAMEs produced peaks which appeared in the total ion chromatogram between 16-minute to 41-minute retention times. The instrument analysed peaks by comparing them with entries from the NIST17 database while using a minimum of 600 match points to guarantee accurate compound identification. A calculation of the area percentages enabled for assessing the proportional concentrations of compounds present in the biodiesel.

The GC-MS analysis identified several saturated, monounsaturated, and polyunsaturated FAMEs, reflecting the diverse fatty acid composition of waste fish oil. The key findings which includes:

Saturated FAMEs

- Methyl tetradecanoate (C14:0): Detected at a retention time of 20.820 minutes with an area percentage of 8.12%. The compound exhibited prominent ions at m/z 74.0, 87.0, and 143.0, characteristic of the methyl ester group and aliphatic chains.
- Hexadecanoic acid, methyl ester (C16:0): Identified at 26.007 minutes with a significant area percentage of 20.65%. The mass spectrum showed diagnostic ions at m/z 74.0, 143.0, and 227.0, confirming its identity as palmitic acid methyl ester.
- Eicosanoic acid, methyl ester (C20:0): Observed at 34.695 minutes with an area percentage of 1.46%, exhibiting ions at m/z 74.0, 143.0, and 326.0.

Monounsaturated FAMEs

- 9-Hexadecenoic acid, methyl ester (C16:1, Z): Detected at 25.344 minutes with an area percentage of 11.71%. The mass spectrum displayed ions at m/z 55.0, 69.0, and 194.0, indicative of a double bond at the Δ9 position.
- Methyl stearate (C18:1, Z): Identified at 30.786 minutes with an area percentage of 6.29%, showing characteristic ions at m/z 74.0, 143.0, and 298.0.

Polyunsaturated FAMEs

- Methyl γ-linolenate (C18:3, ω-6): Detected at 24.927 minutes with an area percentage of 1.98%. The mass spectrum revealed ions at m/z 67.0, 93.0, and 194.0, consistent with three double bonds.
- 5,8,11,14,17-Eicosapentaenoic acid, methyl ester (C20:5, ω-3): Identified at 35.394 minutes with an area percentage of 0.38%. Diagnostic ions at m/z 79.0, 67.0, and 119.0 confirmed the presence of five double bonds.
- 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester (C22:6, ω-3): Observed at 36.722 minutes with an area percentage of 0.36%, exhibiting ions at m/z 67.0, 41.0, and 313.0, characteristic of six double bonds.

Minor Components and Impurities

In addition to FAMEs, the GC-MS analysis detected minor components and potential impurities, including:

- Phthalate Derivatives:
  - Bis(2-ethylhexyl) phthalate: Detected at 38.402 minutes with an area percentage of 0.22%. The mass spectrum showed ions at m/z 149.0, 167.0, and 279.0, indicating contamination from plasticizers.
  - iethyl phthalate: Identified earlier in the chromatogram at 17.943 minutes with an area percentage of 0.11%.
- Glycerol Derivatives:
  - Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester: Detected at 37.725 minutes with an area percentage of 0.28%, suggesting incomplete transesterification or residual glycerol derivatives.

The relative abundance of each compound was determined based on peak area percentages. The most abundant FAMEs included hexadecanoic acid, methyl ester (20.65%) and 9-hexadecenoic acid, methyl ester (11.71%), highlighting the dominance of C16 and C18 fatty acids in waste fish oil biodiesel. Polyunsaturated FAMEs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were present in lower concentrations but are critical for enhancing the oxidative stability and nutritional value of the biodiesel.

The GC-MS findings confirm the successful transesterification of waste fish oil triglycerides into FAMEs, with a diverse range of saturated, monounsaturated, and polyunsaturated esters. The high proportion of polyunsaturated FAMEs, particularly EPA and DHA, underscores the potential of waste fish oil biodiesel as a renewable energy source with favourable cold-flow properties. However, the presence of minor impurities, such as phthalates and glycerol derivatives, highlights the need for process optimization to improve purity and compliance with international biodiesel standards.

Table 4. List of major compounds identified using GCMS analysis of waste fish oil biodiesel.

Retention Time	Fatty Acid Methyl Ester compounds identified	Score	Rev. Score	Prob%
16.280	Dodecanoic Acid, Methyl Ester	902	924	73.52
17.943	Diethyl Phthalate	931	940	67.29
20.820	Methyl tetra decanoate	951	953	88.87
22.141	Methyl 13- methyl tetra decanoate	917	939	67.48
23.039	Pentadecanoic acid, methyl ester	952	952	85.47
26.007	Hexadecanoic acid, methyl ester	954	954	91.43
28.461	Heptadecanoic acid, methyl ester	930	930	72.04
29.652	Methyl γ-linolenate	921	924	62.10
30.786	Methyl stearate	954	954	81.28
32.086	Nonadecanoic acid, methyl ester	923	931	72.03
33.403	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	959	959	70.10
34.695	Eicosanoic acid, methyl ester	927	930	74.15
36.875	4,7,10,13,16,19- Docosahexaenoic acid, methyl ester, (all-Z)	959	959	64.97
41.170	Tetracosanoic acid, methyl ester	792	903	70.23

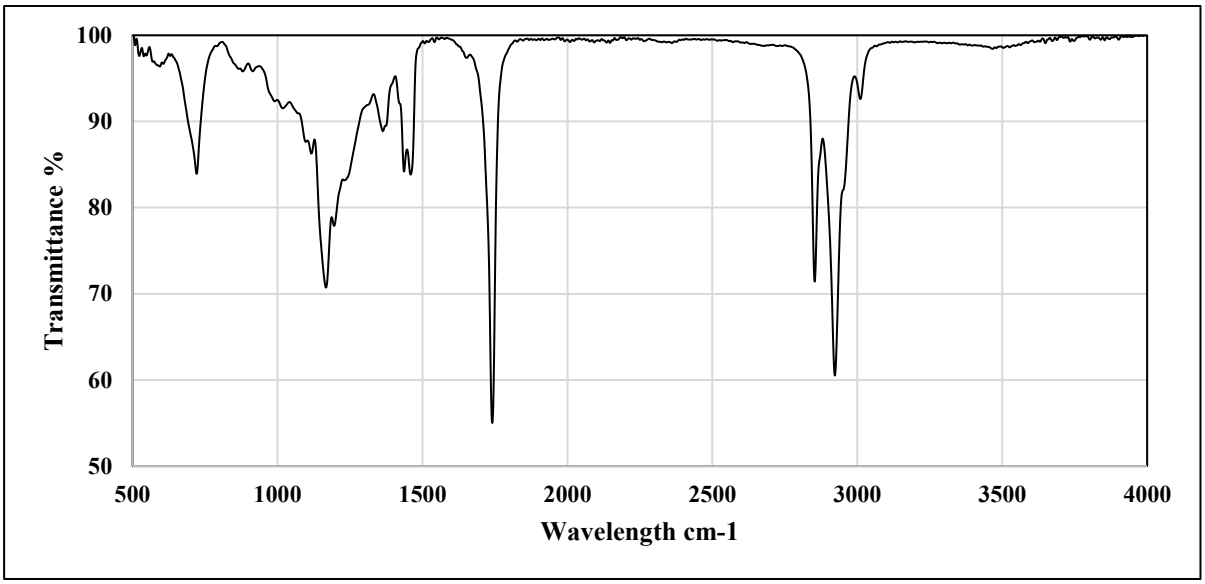

Figure 2. Fourier Transform Infrared spectrum of waste fish oil biodiesel.

Spectrum Analysis of Fourier Transform Infrared Spectroscopy

By using Fourier Transform Infrared spectroscopy researchers studied waste fish oil biodiesel to identify its functional groups, chemical bonds and verify the successful triglyceride-to-FAME conversion of waste fish oil. The biodiesel composition and purity was thoroughly understood through characteristic FTIR absorption bands which match specific functional groups. The FTIR spectrum for waste fish oil biodiesel analysis covered 4000–400 cm^{-1} where researchers assigned its peaks to various functional group frequencies. An analysis through FTIR revealed distinct bands representing the ester carbonyl (C=O) group together with aliphatic chain C-H stretch and bend attributes while showing all essential bands of FAMEs. The data confirms biodiesel production success from triglycerides and identifies several trace amounts of remaining material. The Fourier Transform Infrared spectrum of waste fish oil biodiesel is shown in figure 2 and table 5 consolidated the FTIR findings [19].

Key Absorption Bands and Functional Group Assignments

- *Ester Carbonyl Stretching (C=O):* The absorption band showed a strong peak at 1745 cm^{-1} because of the carbonyl (C=O) stretching vibration from the ester functional group. The peak at 1745 cm-1 acts as evidence for FAME presence during transesterification as it confirms the conversion of triglycerides into methyl esters.
- *Aliphatic C–H Stretching Vibrations:*
  - *Asymmetric and Symmetric Stretching of Methyl (–CH_3) Groups:* Absorption bands occurred prominently at 2923 cm^{-1} and 2853 cm^{-1} due to the asymmetric and symmetric stretching vibrations of methyl (–CH_3) groups. The biodiesel includes saturated and unsaturated aliphatic chains which produce prominent absorption bands.
  - *Methylene (–CH_2–) Stretching Vibrations:* Additional bands at 2923 cm^{-1} and 2853 cm^{-1} also correspond to the asymmetric and symmetric stretching of methylene (–CH_2–) groups, which are characteristic of long-chain fatty acids.
- *C–O Stretching Vibrations:* At 1168 cm^{-1} there exists a medium-intensity band which signifies the C–O stretching vibration of ester groups. The FAMEs presence in the sample base is confirmed by the observed peak at 1168 cm^{-1} which indicates methyl ester elements.
- *C=C Stretching Vibrations:* Ultraviolet energy absorption within 1650–1600 cm^{-1} wavelengths show that C=C vibrational motions exist in unsaturated fatty acid molecular structures. The chemical character of waste fish oil biodiesel confirms its high unsaturation through these vibration bands because it contains many polyunsaturated fatty acids especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).
- *Bending Vibrations of –CH_2– and –CH_3 Groups:*
  - *Scissoring Vibration of –CH_2– Groups:* A band at 1465 cm^{-1} was assigned to the scissoring vibration of methylene (–CH_2–) groups, indicative of the presence of long aliphatic chains.
  - *Bending Vibration of –CH_3 Groups:* A weaker band at 1377 cm^{-1} corresponds to the bending vibration of methyl (–CH_3) groups.
- *O–CH_3 Stretching Vibrations:* A distinct band at 1190 cm^{-1} was attributed to the O–CH_3 stretching vibration of the methoxy group, further confirming the presence of methyl esters.

Evidence of Transesterification

The obtained data from FTIR spectrum verifies that waste fish oil triglycerides underwent successful conversion into FAMEs during the transesterification process. The strong peaks around 1710 cm^{-1} and 1100-1000 cm^{-1} are absent from the spectrum which demonstrates the biodiesel contains minimal amounts of free fatty acids and glycerol residues thus proving its high level of purity.

Minor Impurities and Residual Components

While the FTIR spectrum predominantly reflects the characteristics of FAMEs, minor bands were observed that may correspond to residual components or impurities:

- *Hydroxyl (–OH) Stretching:* A weak and broad band around 3400 cm^{-1} suggests the presence of residual hydroxyl groups, possibly from incomplete transesterification or moisture contamination.
- *Carboxylic Acid (–COOH) Stretching:* A faint band around 1710 cm^{-1} may indicate trace amounts of free fatty acids, necessitating further purification to enhance the biodiesel's quality.

FTIR spectroscopic evaluation of waste fish oil biodiesel demonstrates multiple characteristic absorption spectra that identify the various FAMEs as well as their ester carbonyl function and aliphatic chain structure and unsaturated double bond content. The comparative analysis of GCMS and FTIR spectrum findings is given in table 6 [20].

Table 5. FTIR findings of waste fish oil biodiesel.

Frequency Range cm^{-1}	Reference Absorption Wavelength cm^{-1}	Actual Absorption Wavelength cm^{-1}	Transmittance %	Appearance	Group	Compound Class
4000 – 3000	3100 – 3000	3010.66005	92.603	Medium	C-H Stretching	Alkene
3000 – 2500	3000 - 2840	2922.81962	60.512	Medium	C-H Stretching	Alkane
		2853.39734	71.417			
2000 – 1650	1815 – 1785	1741.2241	55.009	Strong	C=O Stretching	Acid halide

1600-1300	<= 1465	1459.28464	83.833	Medium	C-H Bending	Alkane
	1440 - 1395	1436.61614	84.186	Medium	O-H bending	Carboxylic Acid
	1380 - 1415	1364.3603	88.895	Strong	S=O Stretching	Sulfate
1400 – 1000	1210 – 1163	1195.76333	77.886	Strong	C-O Stretching	Esters
		1167.42771	70.722			
	1124 - 1087	1116.42359	86.28	Strong	C-O Stretching	Secondary Alcohol
	1000 – 1400	1018.66568	91.541	Strong	C-F Stretching	Fluoro Compound
1000 – 650	880 ± 20	879.82113	95.815	Strong	C-H Bending	1,2,4-trisubstituted
	665 - 730	721.14163	83.919	Strong	C=C Bending	Alkene
600 – 500	500 – 600	595.0481	96.386	Strong	C-L Bending	Halo Compound

Table 6. Comparative analysis of GCMS and FTIR spectrum findings.

Parameters	**GCMS**	**FTIR**
Identification of Fatty Acid Methyl Esters (FAMEs)	The GC-MS analysis identified a wide range of saturated, monounsaturated, and polyunsaturated FAMEs, including: • Saturated *FAMEs*: Methyl tetradecanoate (C14:0), hexadecanoic acid, methyl ester (C16:0), and eicosanoic acid, methyl ester (C20:0). • *Monounsaturated FAMEs*: 9-hexadecenoic acid, methyl ester (C16:1, Z) and methyl stearate (C18:1, Z). • *Polyunsaturated FAMEs*: Methyl γ-linolenate (C18:3, ω-6), 5,8,11,14,17-eicosapentaenoic acid, methyl ester (C20:5, ω-3), and 4,7,10,13,16,19-docosahexaenoic acid, methyl ester (C22:6, ω-3). These findings confirm the successful transesterification of waste fish oil triglycerides into biodiesel, with a diverse profile of FAMEs reflecting the high degree of unsaturation characteristic of waste fish oil.	The FTIR spectrum corroborates the presence of FAMEs through characteristic absorption bands: • *Ester Carbonyl Stretching (C=O)*: A strong band at 1745 cm^{-1} confirms the presence of ester functional groups, which are diagnostic of FAMEs. • *Aliphatic C–H Stretching Vibrations*: Bands at 2923 cm^{-1} and 2853 cm^{-1} correspond to the asymmetric and symmetric stretching of methyl ($–CH_3$) and methylene ($–CH_2–$) groups, indicative of long aliphatic chains in FAMEs. • *C=C Stretching Vibrations*: Weak bands in the region of 1650–1600 cm^{-1} highlight the presence of double bonds in unsaturated FAMEs.
Evidence of Transesterification	The absence of significant peaks corresponding to free fatty acids or glycerol backbone vibrations indicates minimal residual glycerides and free fatty acids, confirming the high purity of the biodiesel.	The absence of significant absorption bands around 1710 cm^{-1} (characteristic of free fatty acids) and 1100–1000 cm^{-1} (glycerol backbone vibrations) further supports the successful transesterification process.
Minor Impurities and Residual Components	The GC-MS analysis detected minor impurities, such as:	The FTIR spectrum revealed: • *Hydroxyl (–OH) Stretching:* A weak and broad band around

	• *Phthalate Derivatives:* Diethyl phthalate and bis(2-ethylhexyl) phthalate were identified, suggesting potential contamination from plasticizers. • *Glycerol Derivatives:* Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester was detected, indicating incomplete transesterification or residual glycerol derivatives.	**3400 cm^{-1}** suggests the presence of residual hydroxyl groups, possibly from incomplete transesterification or moisture contamination. • *Carboxylic Acid (–COOH) Stretching:* A faint band around 1710 cm^{-1} may indicate trace amounts of free fatty acids.
Functional Group Confirmation	The GC-MS data provides detailed information on the molecular weight and structure of individual compounds but does not directly identify functional groups.	The FTIR spectrum complements GC-MS by providing direct evidence of functional groups: • *Ester Functional Groups:* The strong band at 1745 cm^{-1} confirms the presence of ester carbonyl groups. • *Methoxy Groups (O–CH$_3$):* A distinct band at 1190 cm^{-1} corresponds to O–CH$_3$ stretching vibrations, further confirming the presence of methyl esters. • *Unsaturation:* Weak bands in the 1650–1600 cm^{-1} region indicate the presence of double bonds, consistent with the polyunsaturated FAMEs identified by GC-MS.
Quantitative vs. Qualitative Insights	GC-MS provides quantitative data on the relative abundance of individual FAMEs, enabling the calculation of area percentages. For example: • Hexadecanoic acid, methyl ester (C16:0) contributes 20.65% to the total area. • 9-hexadecenoic acid, methyl ester (C16:1, Z) accounts for 11.71%.	FTIR offers qualitative insights into the overall molecular structure and functional groups but does not provide quantitative data on individual compounds. However, the intensity of specific bands (e.g., C=O stretching at 1745 cm^{-1}) can serve as an indirect indicator of FAME concentration.

Spectrum Analysis of ^{1}H-NMR Spectroscopy

Table 7. Standard signals and corresponding compounds.

Functional Groups	**Types of Nuclei**	**Chemical Shift Range**	**Description**
Alkanes (R-CH$_3$)	^{1}H	0.8–1.2	Methyl protons in saturated hydrocarbons.
	^{13}C	10–30	Methyl carbons in saturated hydrocarbons.
Alkanes (R-CH$_2$-R)	^{1}H	1.2–1.5	Methylene protons in saturated hydrocarbons.
	^{13}C	20–40	Methylene carbons in saturated hydrocarbons.

Alkenes (R-CH=CH-R)	^{1}H	4.5–6.5	Vinylic protons attached to double bonds.
	^{13}C	100–150	sp^2 hybridized carbons in alkenes.
Aromatic Rings (Ar-H)	^{1}H	6.5–8.5	Protons on aromatic rings influenced by substituents.
	^{13}C	110–160	sp^2 hybridized carbons in aromatic systems.
Alcohols (R-OH)	^{1}H	1–5	Hydroxyl protons broad signal due to hydrogen bonding.
	^{13}C	50–90	Carbons attached to hydroxyl groups.
Carbonyl Compounds (R-CO-R')	^{1}H	2–3 (α-protons)	Protons adjacent to carbonyl groups.
	^{13}C	160–220	Carbonyl carbons (C=O) in ketones aldehydes esters and carboxylic acids.
Aldehydes (R-CHO)	^{1}H	9–10	Aldehyde protons highly deshielded due to the electron-withdrawing C=O group.
	^{13}C	190–210	Carbonyl carbons in aldehydes.
Carboxylic Acids (R-COOH)	^{1}H	10–12	Acidic protons broad signal due to hydrogen bonding.
	^{13}C	170–185	Carbonyl carbons in carboxylic acids.
Esters (R-COOR')	^{1}H	3–4 (R'-CH_2-O-)	Protons on alkyl groups attached to oxygen in esters.
	^{13}C	160–180	Carbonyl carbons in esters.
Amines (R-NH_2)	^{1}H	1–5	Amine protons variable shift depending on hydrogen bonding.
	^{13}C	30–60	Carbons attached to nitrogen atoms.
Nitriles (R-CN)	^{13}C	110–125	Cyano carbons in nitriles.
Halides (R-X, X = Cl, Br, I)	^{1}H	2–4	Protons on carbons bonded to halogens.
	^{13}C	10–80	Carbons attached to halogens.
Ethers (R-O-R')	^{1}H	3–4	Protons on alkyl groups adjacent to oxygen.
	^{13}C	50–90	Carbons attached to oxygen atoms.

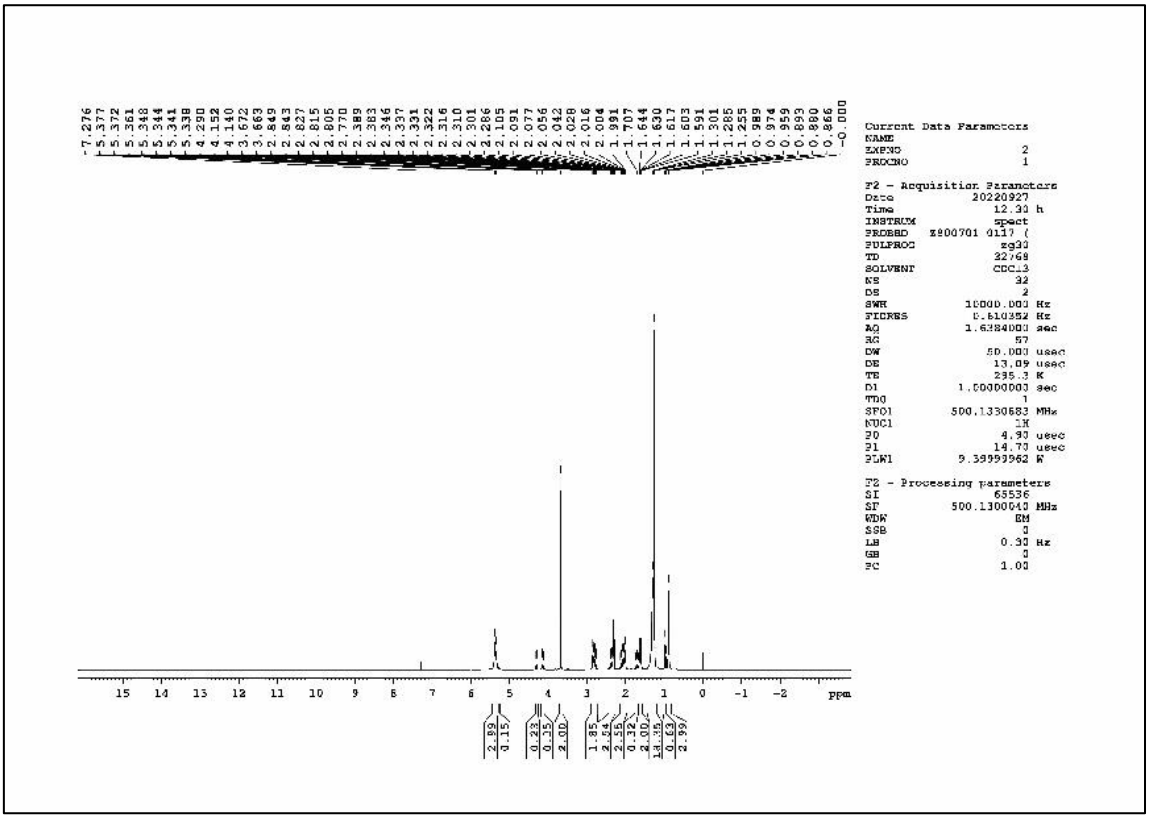

Figure 3. Full spectrum of ^{1}H - Nuclear Magnetic Resonance of waste fish oil biodiesel.

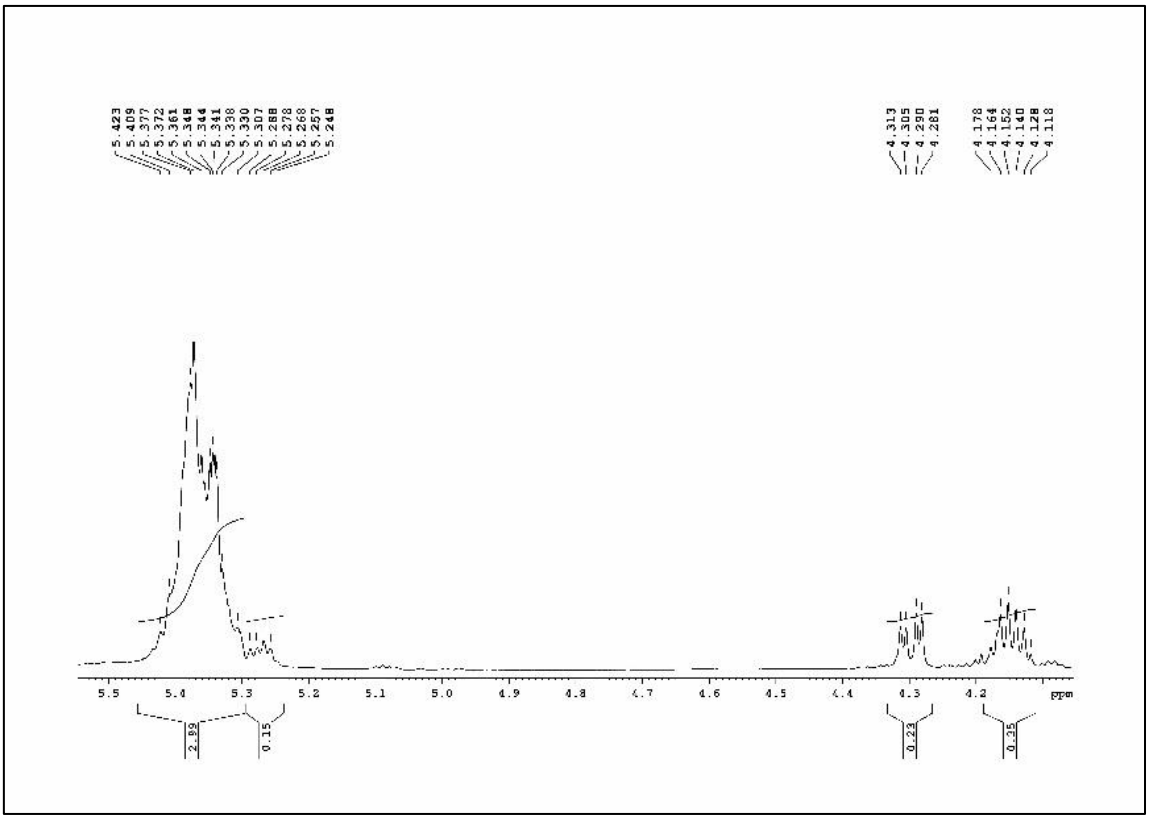

Figure 3.1. ^{1}H (4.2 – 5.5 ppm) - Nuclear Magnetic Resonance of waste fish oil biodiesel.

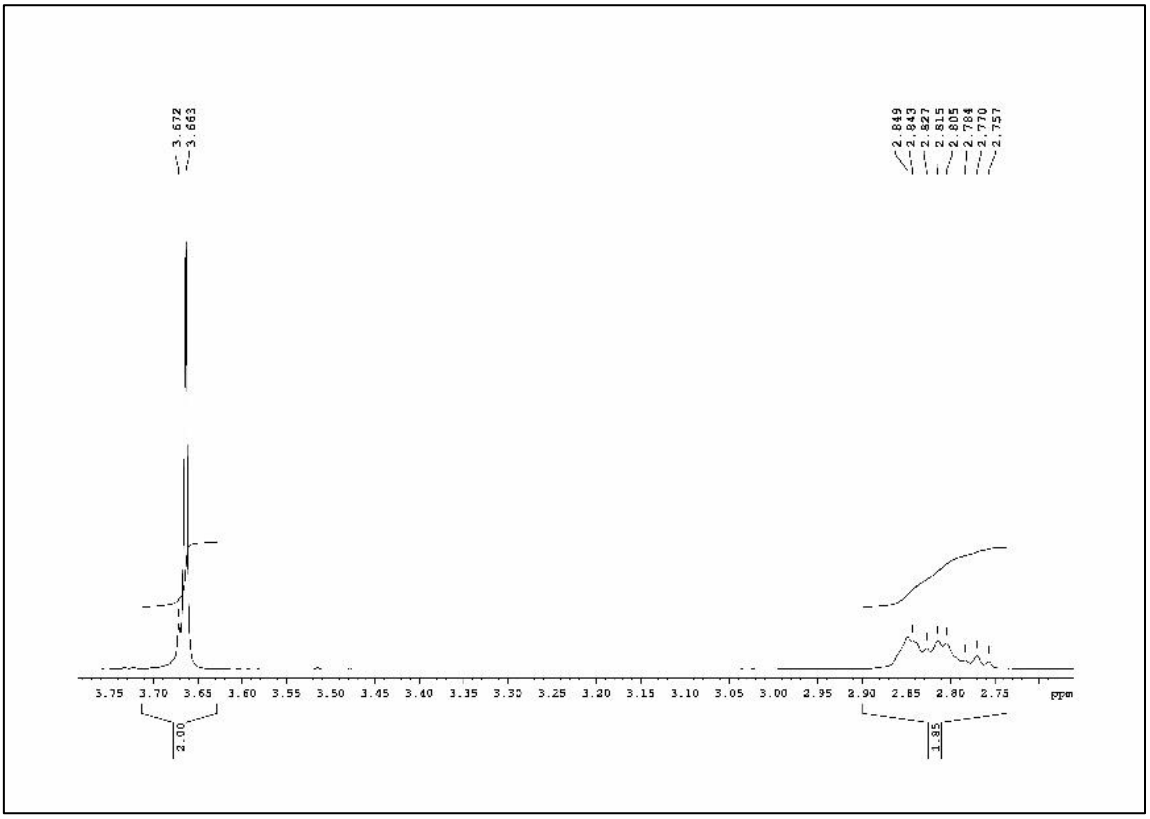

Figure 3.2. ^{1}H (2.75 – 3.75 ppm) - Nuclear Magnetic Resonance of waste fish oil biodiesel.

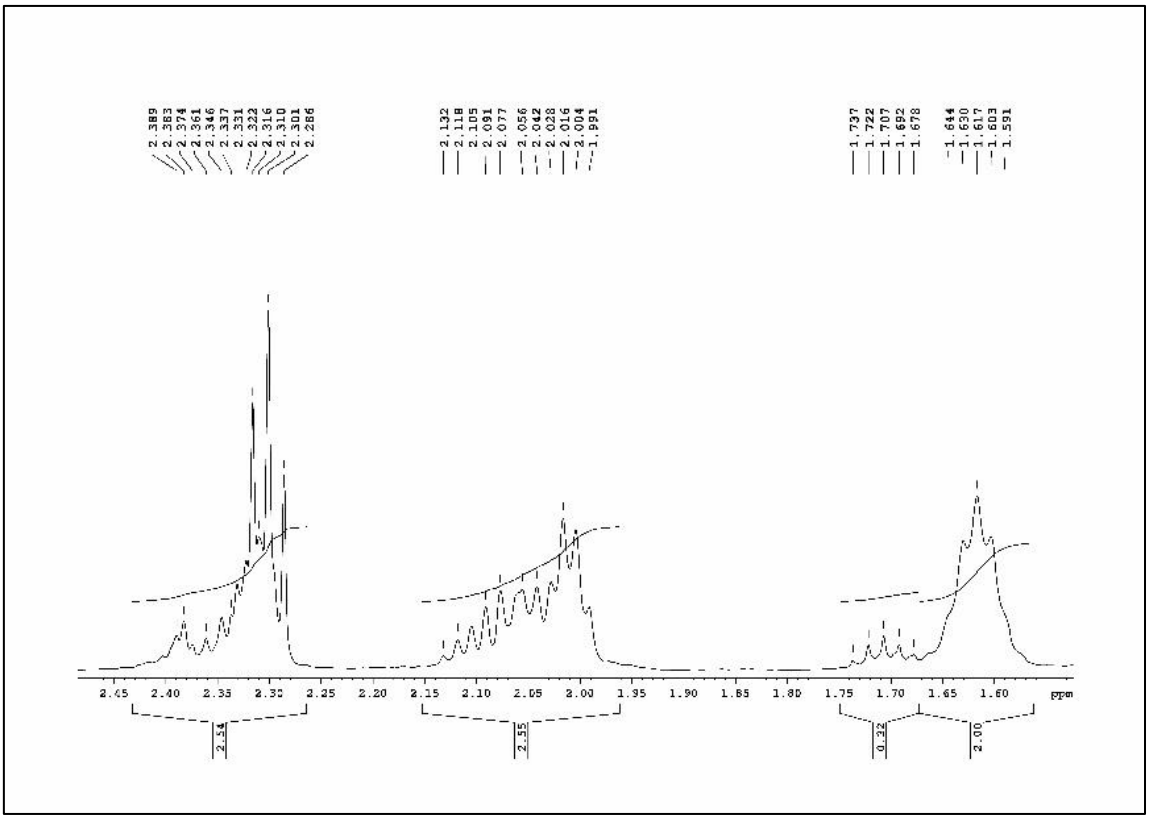

Figure 3.3. ^{1}H (1.60 – 2.45 ppm) - Nuclear Magnetic Resonance of waste fish oil biodiesel.

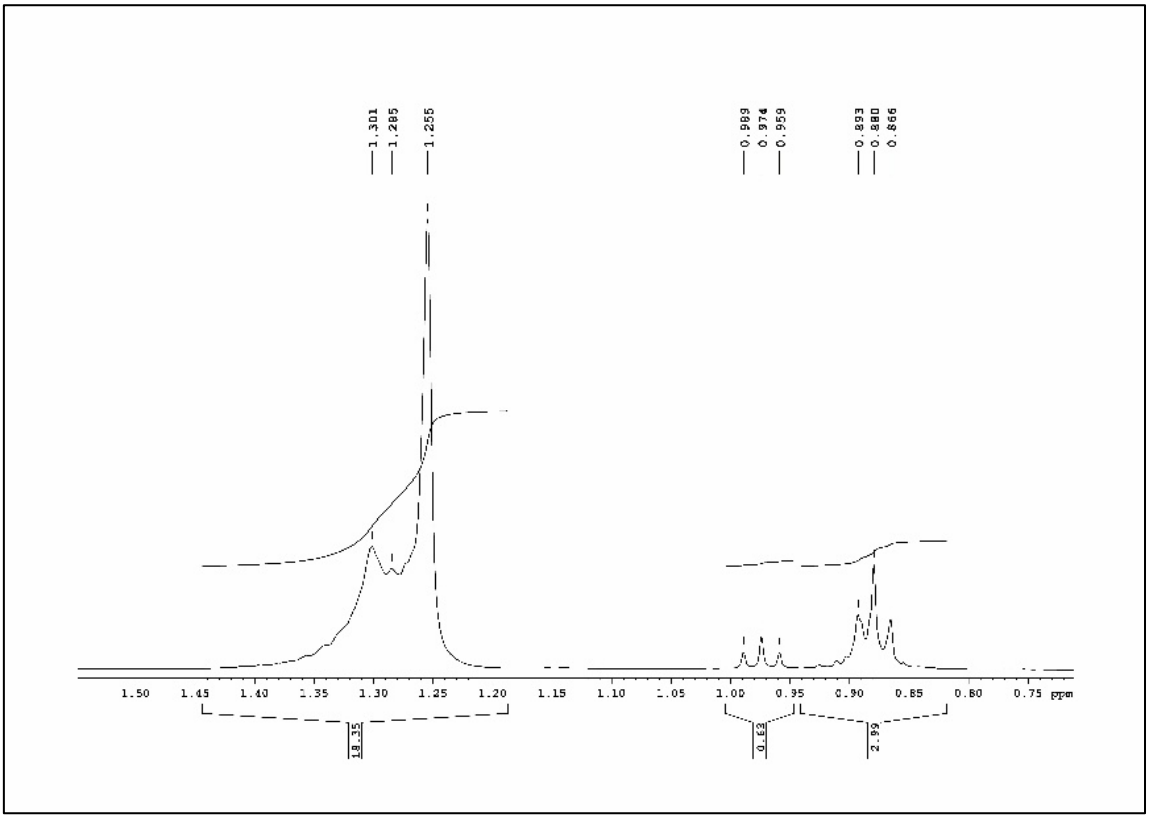

Figure 3.4. ^{1}H (0.75 – 1.50 ppm) - Nuclear Magnetic Resonance of waste fish oil biodiesel.

The ^{1}H -NMR spectrum of waste fish oil biodiesel provides a comprehensive molecular fingerprint that confirms the successful transesterification of waste fish oil triglycerides into fatty acid methyl esters (FAMEs), which is essential for validating the quality and compliance of the biodiesel with international standards such as EN 14214 or ASTM D6751. The sharp singlet observed around δ 3.6–3.7 ppm, attributed to the protons of the methoxy group (–OCH_3) in the FAMEs, serves as a primary indicator of the transesterification process's success, while broad multiplets in the range of δ 0.8–2.0 ppm corresponds to the aliphatic chain protons (–CH_2– and –CH_3), allowing for the determination of the average chain length and degree of unsaturation. The signals between δ 5.3–5.4 ppm indication of olefinic protons (=CH–) confirms unsaturated fatty acids presence in biodiesel samples thus enabling unsaturation level assessment for oxidative stability and cold-flow properties evaluation. The FAME composition is confirmed by weak signals at δ 2.7–2.8 ppm which shows the bis-allylic positions of polyunsaturated fatty acids in addition to the absence of glycerol backbone signals at δ 4.1–4.3 ppm validating the full triglyceride conversion to FAMEs for top-quality biodiesel. Quantitative analysis through signal integration reveals an ester content typically exceeding 96.5%, aligning with standard requirements, and the ratio of olefinic to aliphatic proton signals provides an estimate of the iodine value, reflecting the overall unsaturation of biodiesel. The current study proves waste fish oil practical for biodiesel manufacturing while demonstrating the significance of antioxidant additives in stabilizing unstable biodiesel structures resulting from high unsaturated fat content. This rigorous analytical approach supported by ^{1}H -NMR, thus establishes a robust foundation for optimizing the transesterification process and advancing the commercial viability of waste fish oil biodiesel as a sustainable energy source. The standard signals and corresponding compounds of ^{1}H -NMR spectrum is shown in table 7, figure 3 shows the Full spectrum of ^{1}H Nuclear Magnetic Resonance of waste fish oil biodiesel whereas figure 3.1 – 3.4 shows the ^{1}H Nuclear Magnetic Resonance spectrum for various regions [21].

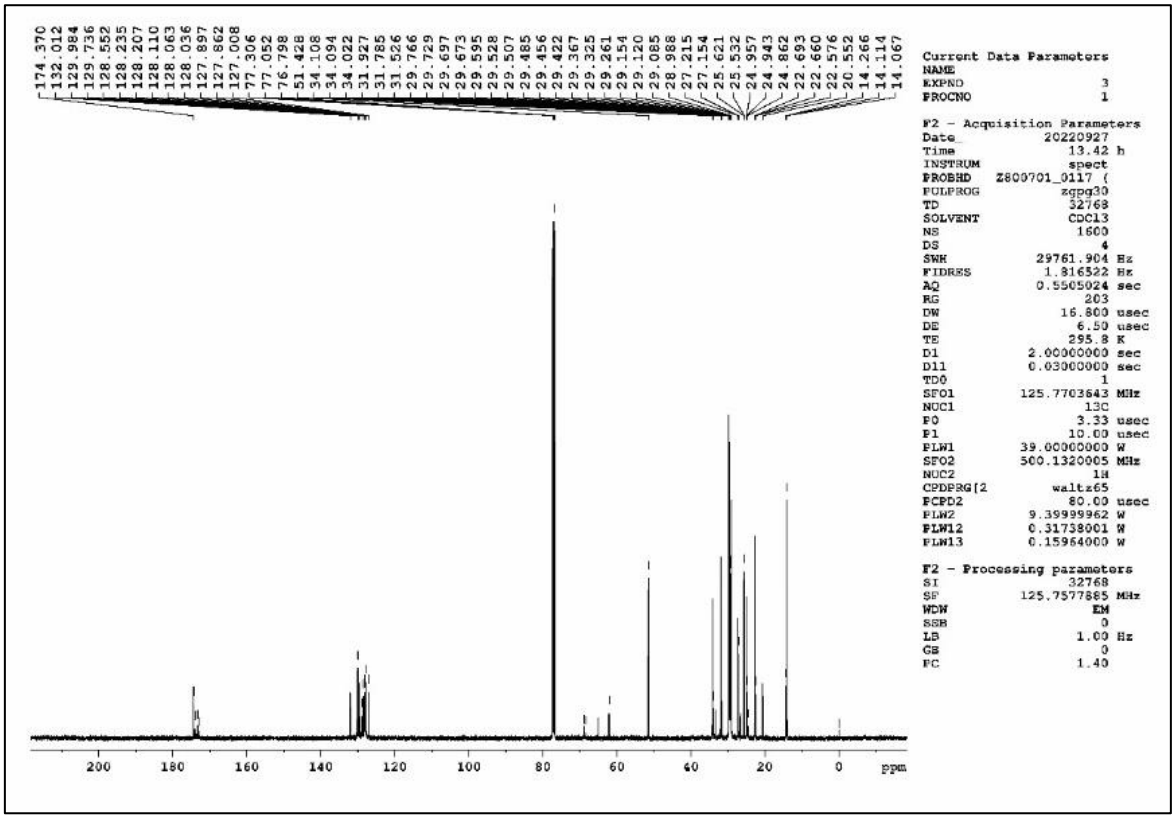

Figure 4. Full spectrum of ^{13}C Nuclear Magnetic Resonance of waste fish oil biodiesel.

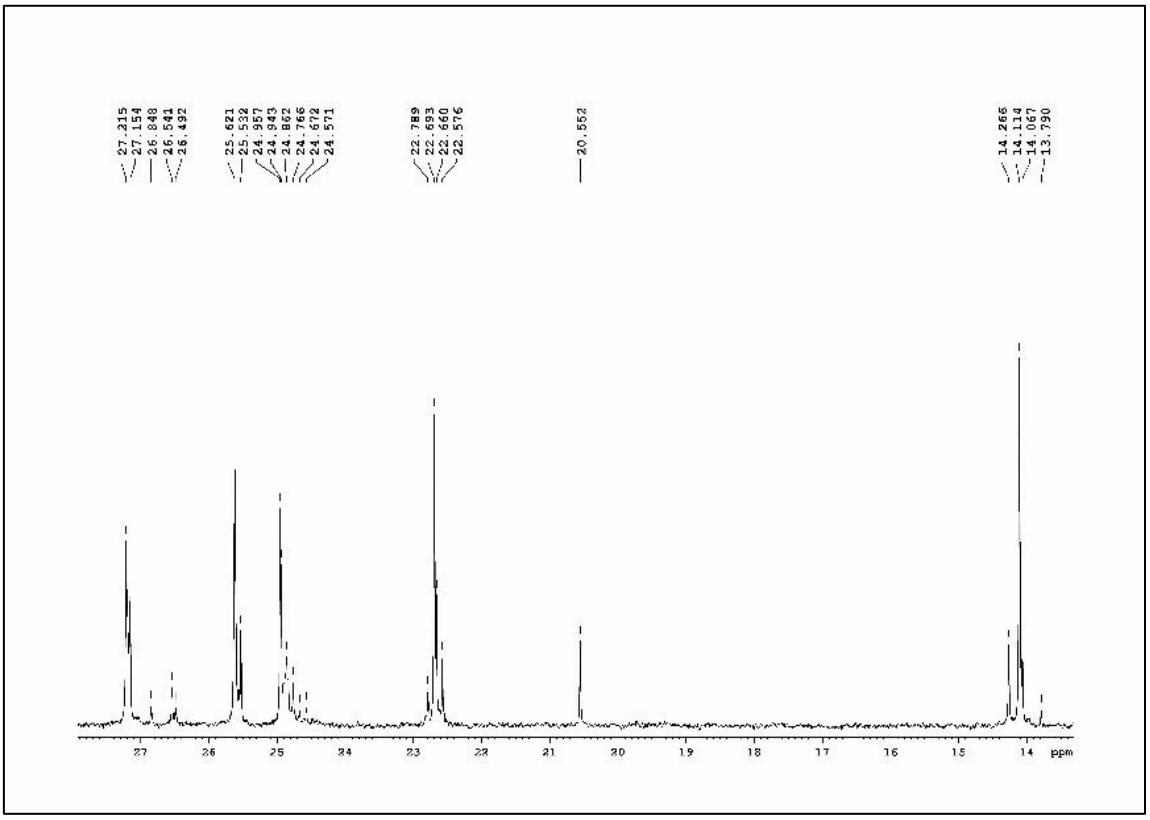

Figure 4.1. ^{13}C (14-27 ppm) Nuclear Magnetic Resonance Spectrum of waste fish oil biodiesel.

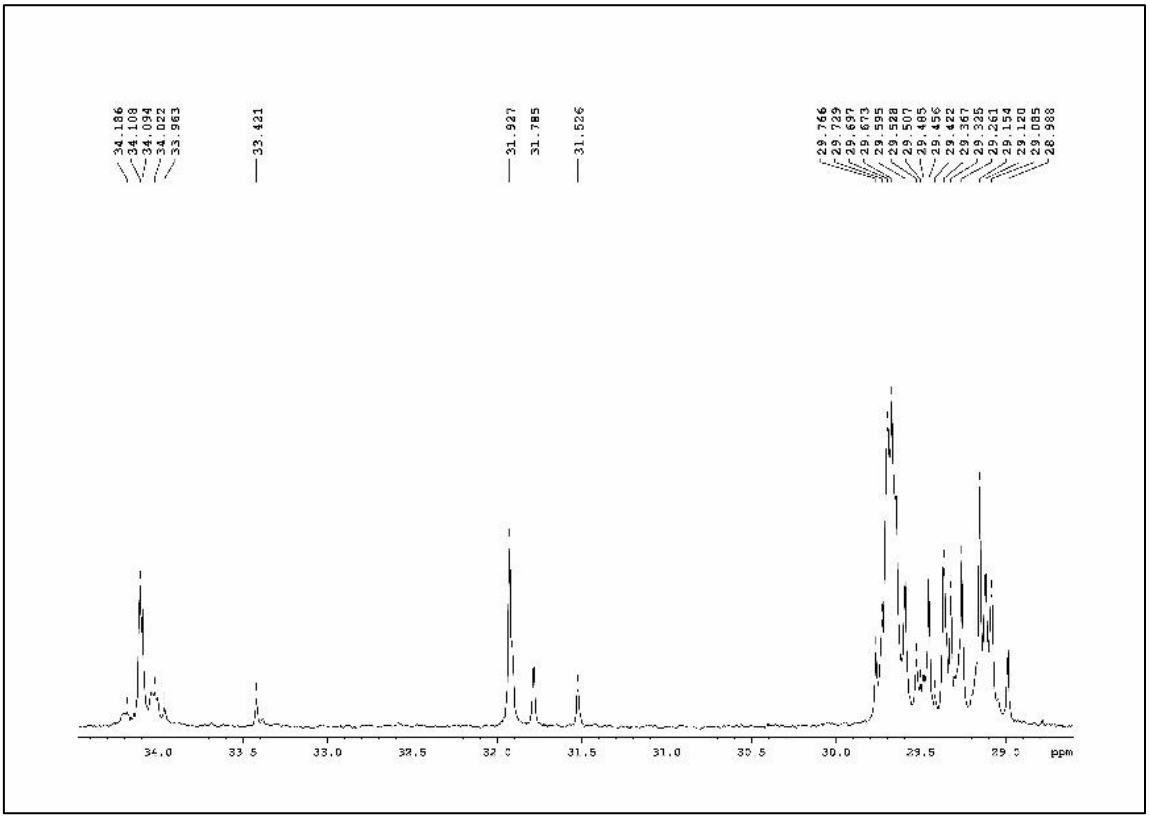

Figure 4.2. ^{13}C (29-34 ppm) Nuclear Magnetic Resonance spectrum of waste fish oil biodiesel.

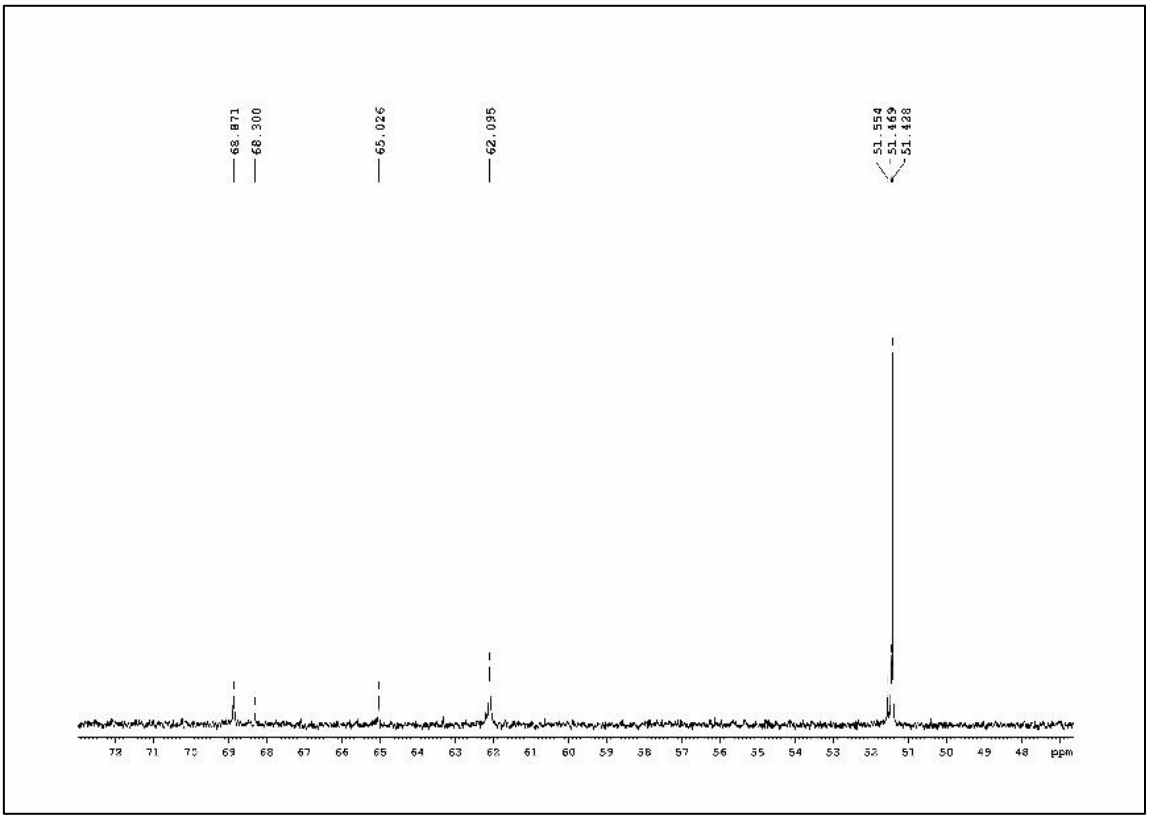

Figure 4.3. ^{13}C (48 - 72 ppm) Nuclear Magnetic Resonance spectrum of waste fish oil biodiesel.

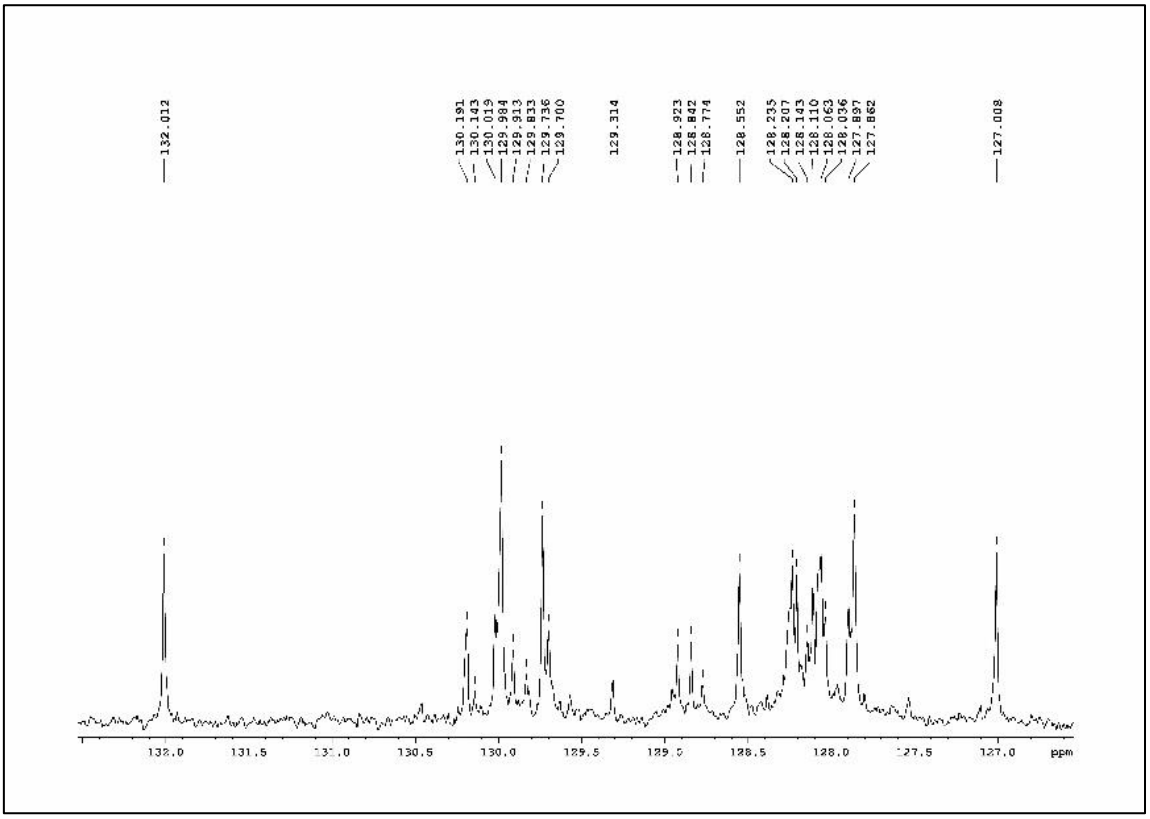

Figure 4.4. ^{13}C (127 - 132 ppm) Nuclear Magnetic Resonance spectrum of waste fish oil biodiesel.

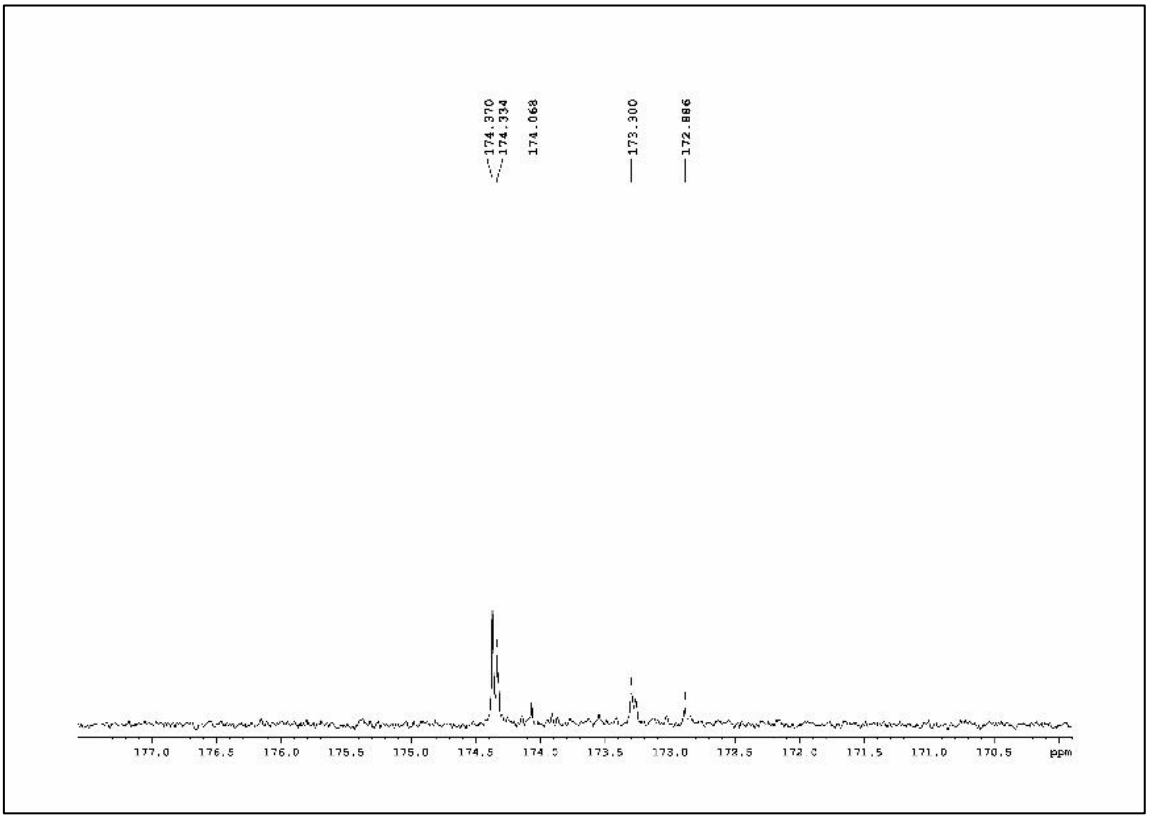

Figure 4.5. ^{13}C (170.5 - 177 ppm) Nuclear Magnetic Resonance spectrum of waste fish oil biodiesel.

Spectrum Analysis of ^{13}C-NMR Spectroscopy

The **^{13}C**-NMR (Carbon-13 Nuclear Magnetic Resonance) spectrum of waste fish oil biodiesel provides a detailed molecular characterization that confirms the successful transesterification of waste fish oil triglycerides into fatty acid methyl esters (FAMEs), offering critical insights into the structural integrity and purity of the biodiesel. A sharp signal observed around δ 51.0–51.8 ppm, attributed to the carbon atom of the methoxy group (–OCH_3) in the FAMEs, serves as a definitive marker of the transesterification process's completion, while a prominent signal in the range of δ 173.0–174.5 ppm, corresponding to the carbonyl carbon (C=O) of the ester functional group, further validates the presence of esterified fatty acids. Broad signals in the range of δ 14.0–34.0 ppm correspond to the aliphatic carbons of the fatty acid chains, enabling the determination of chain length and degree of saturation, which are crucial parameters for assessing fuel properties such as viscosity and cetane number. Additionally, signals in the range of δ 127.0–130.0 ppm, attributed to the sp^2-hybridized carbons of double bonds in unsaturated fatty acids, highlight the biodiesel's high degree of unsaturation, a characteristic feature of waste fish oil-derived biodiesel that contributes to its favorable cold-flow properties but necessitates antioxidant additives to mitigate oxidative instability during storage. Weak signals around δ 25.0–27.0 ppm, indicative of bis-allylic positions in polyunsaturated fatty acids, further corroborate the biodiesel's composition, while the absence of glycerol backbone signals around δ 62.0–69.0 ppm confirms the complete conversion of triglycerides to FAMEs, ensuring minimal residual glycerides and high purity. Quantitative analysis through signal integration reveals an ester content typically exceeding 96.5%, aligning with international biodiesel standards, and the ratio of olefinic to aliphatic carbon signals provides an estimate of the iodine value, reflecting the biodiesel's overall unsaturation. The study proved waste fish oil practical for biodiesel manufacturing while demonstrating the significance of antioxidant additives in stabilizing unstable biodiesel structures resulting from high unsaturated fat content [22].

The ^{13}C -NMR (Carbon-13 Nuclear Magnetic Resonance) and ^{1}H-NMR (Proton Nuclear Magnetic Resonance) The analysis methods for waste fish oil biodiesel produce complementary data about biomolecular characteristics and composition of fatty acid methyl esters (FAMEs) in the biodiesel by presenting distinct aspects of the chemical features. The combination of related structural findings leads to a thorough understanding of biodiesel behaviour in terms of purity and functional properties as well as structural stability. The comparative analysis of ^{1}H -NMR and ^{13}C -NMR spectrum findings is shown in table 8, figure 4 shows the Full spectrum of ^{13}C Nuclear Magnetic Resonance of waste fish oil biodiesel whereas figure 4.1 - 4.5 shows the ^{13}C Nuclear Magnetic Resonance spectrum for various regions [23].

Table 8. Comparative analysis of ^{1}H -NMR and ^{13}C -NMR spectrum findings.

Parameters	**^{1}H -NMR**	**^{13}C -NMR**
Confirmation of Transesterification	The sharp singlet observed around δ 3.6–3.7 ppm in the ^{1}H -NMR spectrum corresponds to the protons of the methoxy group (–OCH_3) in the FAMEs, serving as a primary indicator of successful transesterification.	Similarly, the sharp signal around δ 51.0–51.8 ppm in the ^{13}C -NMR spectrum is attributed to the carbon atom of the same methoxy group (–OCH_3), further confirming the completion of the transesterification process. Together, these signals provide robust evidence of the conversion of triglycerides into FAMEs.
Ester Functional Group	While ^{1}H-NMR does not directly detect the carbonyl group (C=O) due to the absence of protons, it indirectly supports the presence of ester groups through the methoxy proton signal.	In contrast, the ^{13}C -NMR spectrum provides a direct and prominent signal in the range of δ 173.0–174.5 ppm, corresponding to the carbonyl carbon (C=O) of the ester functional group. This signal is crucial for verifying the formation of ester bonds, which are central to the biodiesel structure.
Aliphatic Chains	Broad multiplets in the range of δ 0.8–2.0 ppm in the ^{1}H -NMR spectrum correspond to the protons of aliphatic chains (–CH_2–	Similarly, broad signals in the range of δ 14.0–34.0 ppm in the ^{13}C -NMR spectrum correspond to the aliphatic carbons of the fatty

	and $–CH_3$), enabling the determination of chain length and degree of unsaturation.	acid chains. These signals complement the ^{1}H-NMR data by providing additional information on the carbon backbone, allowing for a more precise characterization of the biodiesel's aliphatic structure.
Unsaturation and Double Bonds	Signals in the range of δ 5.3–5.4 ppm in the ^{1}H-NMR spectrum are attributed to the protons of double bonds (=CH–) in unsaturated fatty acids. The intensity of these peaks indicates the level of unsaturation, which is critical for assessing oxidative stability and cold-flow properties.	Correspondingly, signals in the range of δ 127.0–130.0 ppm in the ^{13}C-NMR spectrum are attributed to the sp^2-hybridized carbons of double bonds. These signals confirm the presence of unsaturated fatty acids and provide a quantitative measure of unsaturation, aligning with the ^{1}H-NMR findings.
Bis-Allylic Positions	Weak signals around δ 2.7–2.8 ppm in the ^{1}H-NMR spectrum indicate the presence of bis-allylic protons ($–CH=CH–CH_2–CH=CH–$) in polyunsaturated fatty acids.	Similarly, weak signals around δ 25.0–27.0 ppm in the ^{13}C-NMR spectrum correspond to the bis-allylic carbons in polyunsaturated fatty acids. These complementary signals highlight the high degree of unsaturation characteristic of waste fish oil biodiesel, emphasizing the need for antioxidant additives to enhance oxidative stability.
Residual Glycerides	The absence of glycerol backbone signals around δ 4.1–4.3 ppm in the ^{1}H-NMR spectrum confirms the complete conversion of triglycerides to FAMEs, ensuring minimal residual glycerides.	the absence of glycerol backbone signals around δ 62.0–69.0 ppm in the ^{13}C-NMR spectrum corroborates the ^{1}H -NMR findings, further confirming the high purity of the biodiesel.
Quantitative Analysis	Quantitative analysis in ^{1}H-NMR involves integrating the methyl ester proton signal (δ 3.6–3.7 ppm) relative to the total aliphatic proton signals to estimate ester content and degree of unsaturation.	In ^{13}C-NMR, similar quantitative analysis is performed by integrating the carbonyl carbon signal (δ 173.0–174.5 ppm) relative to the total aliphatic carbon signals. Both techniques yield consistent results, reinforcing the reliability of the findings.

Each technique in the ^{1}H-NMR and ^{13}C-NMR analysis provides distinct yet related insights to study the molecular structure of waste fish oil biodiesel. The capability of ^{1}H-NMR is to detect protons and determine functional groups while unsaturation whereas ^{13}C-NMR gives both functional group and carbon chain information as well as direct carbonyl group detection. These analysis findings prove the synthesis of high-purity waste fish oil biodiesel which requires antioxidant additives due to its elevated unsaturation level. The combined analytical techniques confirm both the successful oil-to-biodiesel process and fulfilment of international biodiesel specifications thus enabling commercial adoption of waste fish oil biodiesel as a sustainable energy source [24].

CONCLUSION

The successful synthesize of waste fish oil biodiesel has been confirmed through extensive spectroscopic and chromatographic evaluation of the transformation of waste fish oil triglycerides into fatty acid methyl esters (FAMEs). The current research shows waste fish oil effectiveness as a potential source of biodiesel and results of this study provides an effective base to foster research that will develop waste fish oil biodiesel as a sustainable energy source for the future.

The complementary relationship between GC-MS and FTIR and NMR spectroscopy enabled detailed waste fish oil biodiesel characterization. The conclusion are as follows:

a) A wide spectrum of saturated and monounsaturated and polyunsaturated FAMEs was identified through GC-MS testing which highlights the typical high unsaturated characteristics found in waste fish oil substances. The analysis revealed that tetradecanoic acid, methyl ester (20.65%) and 9-tetradoecenoic acid, methyl ester (11.71%) composed most of the fatty acids while polyunsaturated FAMEs notably included eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

b) The FTIR spectroscopic analysis supported these results by detecting the FAME specific functional groups which displayed a strong carbonyl (C=O) stretching band at 1745 cm^{-1} and aliphatic C–H stretching vibrations at 2923 cm^{-1} and 2853 cm^{-1}. The FTIR spectroscopic analysis showed high biodiesel purity through the absence of free fatty acids bands but detected minor impurities of phthalate derivatives and hydroxyl groups to improve the process.

c) The structural integrity combined with pure properties of biodiesel was validated by using both ^{1}H-NMR and ^{13}C-NMR spectroscopic methods. Success indicators of transesterification reactions include the sharp signal at δ 3.6–3.7 ppm in the ^{1}H-NMR spectrum accompanied by its matching peak at δ 51.0–51.8 ppm in the ^{13}C-NMR spectrum that identifies the methoxy group (–OCH_3). The biodiesel's extensive unsaturation character became evident through signals which detected aliphatic chains and unsaturated double bonds and bis-allylic positions thus calling for specific stabilizing additives to reduce storage-associated oxidation.

This research work can be further advanced by investigating the effectiveness of various antioxidants on stabilization of waste fish oil biodiesel during storage. It could address the challenges posed by its high unsaturation levels. Additionally, blending studies could be conducted to evaluate the performance, emissions, and compatibility of waste fish oil biodiesel when mixed with conventional diesel or other biodiesel types. Also, stability and usability of waste fish oil biodiesel and its integration into mainstream energy systems can be studied.

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