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Marine Oils as Potential Feedstock for Biodiesel Production: Physicochemical Characterization

Deepika Dave*, Vegneshwaran V Ramakrishnan, Sheila Trenholm, Heather Manuel, Julia Pohling and Wade Murphy

Centre for Aquaculture and Seafood Development, Marine Institute of Memorial University of Newfoundland, St. John's, Newfoundland, Canada

Abstract

Physico-chemical characteristics of four crude marine oils including farmed salmon, seal, cod liver and wild salmon are compared and interpreted with regard to their suitability as biodiesel feedstock. The physico-chemical properties including specific gravity, pH, ash content, acid value, iodine value, saponification value, p-anisidine value, peroxide value, TOTOX value, free fatty acid, flash point, kinematic viscosity, refractive index, lipid classes and fatty acid classification of all four marine oils were evaluated. The characterized marine oils were pale yellow to orange in color and stable in the liquid state at room temperature. The pH (6.5-6.8) values of all oils were neutral. The specific gravity (0.921-0.924 g/cm^3), water content (179-325 ppm), ash content (0.0027-0.00455%), free fatty acids (0.03-1.23%), acid value (0.057-0.771 mg KOH/g), peroxide value (5.13-9.17 meq O_2/kg oil) and p-anisidine value (3.36-9.67) of all oils were within recommended limits except for higher water content in the seal oil (689 ppm), higher acid value in farmed salmon (2.441 mg KOH/g) and seal oil (0.958 mg KOH/g) and higher iodine value (116-139.15 g I_2/100 g). A drying step needs to be implemented for the removal of water as it can lead to corrosion of internal combustion engine components. Due to higher iodine values, all the oils were drying oils except farmed salmon oil which was semidrying oil and susceptible to become rancid which causes reduction of pour point of biodiesel produced in the absence of antioxidant. All four marine oils were more likely to polymerize in the heat of the engine if used directly without transesterification. Flash point of all marine oils were above 200°C so, there is no risk of fire outbreaks in case of accidents. Due to higher triacylglycerol (81-93%) content, all the marine oils are suitable as a feedstock for biodiesel production via transesterification. Cod liver (14.72%) and wild salmon oil (9.92%) were rich in polar lipids while the farmed salmon (2.43%) and wild salmon (2.43%) were low in polar lipids. The phospholipids (1.21-1.67%) were higher than the recommended limit of ≤10 ppm so degumming process is required prior to biodiesel production. All the marine oils in this study have a high degree of unsaturation and polyunsaturated fatty acids and therefore the biodiesel produced from all oils will have less oxidation stability and result in the precipitation of the biodiesel components in a fuel feeding system or combustion chamber. Therefore, it is essential to stabilize the oil using an antioxidant immediately after extraction/production to obtain a high quality biofuel.

Keywords: Marine oil; Biofuel; Feed stock

Introduction

The continuously increasing demand for energy has been translated into increased cost of crude oils, shortage of fossil fuels and intensified emission of greenhouse gases worldwide. Adverse environmental concerns due to use of non-renewable fossil fuel and the rapid decline of their reserves are the two largest incentives to find energy alternatives that are renewable and unlimited [1-4]. Renewable energy resources of biological origin (biofuels) have smaller net greenhouse gas emissions. Currently, biodiesel and bioethanol production are gaining momentum all across the globe due to the shrinking supply of oil reserves, security of source, cost of production and the impending threat of global warming [4-6]. However, sustainable production of biofuels will require a resourceful biomass conversion process.

Biodiesel is a biofuel that is obtained from plant and marine oils or animal fats. Biodiesel, as a diesel-equivalent, has a potential share among biofuels of about three quarter of all refinery distillate fuel oils [7-9]. One of the biggest challenges in biodiesel production is the availability of feed-stock. There is a concern about using plant derived oils and fats since the crops used for biodiesel production are also needed for food, feed and oleochemical industries [10,11]. Biodiesel factories must compete with food, cosmetic, chemical and livestock feed demands. There is also an environmental concern because an increased demand for vegetable oils requires an increase in the use of fertilizers which contribute to greenhouse gas emissions. In fact, biodiesel production from heavily fertilized crops could result in a 70% increase (from the current value) in greenhouse gas emissions [11-13].

Canada's commercial fishing industry is valued at approximately $3 billion a year with its aquaculture industry worth $845 million. Of the 799,567 tons marine landings and 163, 036 tons aquaculture in 2012, 34% and 14%, respectively came from Newfoundland and Labrador [14]. There are 187 registered fish processing facilities in Newfoundland, ranging in size from feeder plants (processing fish to the fillet) to large year-round plants (processing fish into various fresh and frozen products including secondary processing) [15]. Of the 145 licensed aquaculture plants, 84 produced salmonids [16]. In 2012, salmonid, cod and seal production accounted 16,831 tons (79.2%), 8334 tons (3.2%) and 67,567 (26.3%), respectively and was valued at $90.1 million [17]. Processing of fish generates large amounts of solid wastes, up to 30-80% of the body weight of the processed fish. Currently, most of the fish processing waste is dumped at sea or in landfills [9].

Sealing has thrived in Newfoundland and Labrador for hundreds of

***Corresponding author:** Deepika Dave, Centre for Aquaculture and Seafood Development, Marine Institute of Memorial University of Newfoundland, St. John's, Newfoundland, Canada, Tel: 709-757-0732; E-mail: Deepika.Dave@mi.mun.ca

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years and the sealing industry has been worth over $55 million per year to the province's economy [18]. Only relatively unrefined seal products (raw seal skins, seal oil and seal meat) can be identified in Canada's export statistics [19]. While the focus is directed on the method used for killing seal, much of the negativity arises from the utter waste of the resource and environmental impact of discarding seal carcasses into the ocean. This is largely due to the lack of economic feasibility to convert these waste materials into high value added products.

Newfoundland and Labrador's seafood industry generates on the average 102,850 tons (25% of Canada's fish waste) of marine wastes (processing discards). Currently, most of the processing waste is bulk packaged and sold to mink producers and/or pet food manufacturers in eastern Canada. A small percentage of the processing discards (gut material) and morts are bulk shipped to a composting operation in the Corner Brook area. There is a planned expansion of Newfoundland salmon aquaculture up to 50,000 metric tons in the next 5 years which will increase the volume of waste generated from aquaculture processing operations and mortalities. Currently, NL has limited capacity to handle this waste in terms of composting, mink feed, rendering and landfilling [15]. Thus, it is important to develop by-product applications that demand large volumes of marine waste, in order to make these industries viable, greener and environmentally friendly. Newfoundland and Labrador has the potential to produce 2600 tons of marine oils extracted from seal harvesting and fish processing waste as the largest potential source of biodiesel feedstock [20]. Converting marine oil into biodiesel would benefit the marine industry sectors in reducing the disposal cost of these wastes to landfills and utilize biodiesel for operating feed barges, marine vessels and generators located at their remote locations.

The properties of marine oils are less uniform compared to fresh vegetable oils because of the physical and chemical changes mainly due to oxidative and hydrolytic reactions that take place during handling, stabilization, storage and oil extraction process [21]. The initial evaluation of the physical and chemical composition of the feed stock oil is very essential for qualitative identification prior to utilization in biodiesel production. Feed stock for biodiesel production are given priority selection and pre-treatment according to the level of free fatty acids, impurities, water content, ash content, acid value, iodine value, saponification value, p-anisidine value, peroxide value, free fatty acid, flash point, kinematic viscosity and refractive index [22-25].

In the present study, the physico-chemical characteristics of four crude marine oils including farmed salmon, seal, cod liver and wild salmon are compared and interpreted with regard to their suitability as biodiesel feedstock.

Materials and Methods

This study was carried out at the Marine Bioprocessing Facility of the Centre for Aquaculture and Seafood Development, Marine Institute of Memorial University of Newfoundland in St. John's, Newfoundland, Canada. Fatty Acid profiling was performed at the Oceans Sciences Centre of Memorial University of Newfoundland, Logy Bay, Newfoundland, Canada.

Chemicals and reagents

Reagent 'A' (Calcium Hydride) and Reagent 'B' were obtained from Sandy Brae Laboratories (Wilmington, Delaware, USA). Buffer Solutions (pH 4.0, 7.0 and 10.0), sodium thiosulfate, cyclohexane and potassium hydroxide were obtained from Sigma-Aldrich (Oakville, Ontario, Canada). Wijs iodine solution, iso-octane (2,2,4-trimethylpentane), glacial acetic acid (Ricca), concentrated sulfuric acid (Acros Organic), para-anisidine (Acros Organic), hydrochloric acid (concentrated), soluble starch and chloroform were obtained from Fisher Scientific (Ottawa, Ontario, Canada). Potassium iodide (BDH), potassium dichromate (BDH), and formic acid (BDH) were obtained from VWR International (Mississauga, ON, Canada). Ethanol (95%) was obtained from East Chem (Paradise, NL, Canada).

Marine oils

Crude cod (*Gadus morhua)* liver and pacific salmon *(Oncorhynchus)* oil were purchased from J. Edwards International Inc. (a bulk oil provider, Quincy, MA, USA). Crude Atlantic salmon *(Salmo salar)* and Harp seal *(Pagophilus groenlandicus)* oils were purchased from an oil extraction plant in Newfoundland, operated by Barry Group Inc. Salmon and seal oil were extracted from the waste stream from salmon guts and seal blubber, respectively that were obtained from local processing plants Both oils were extracted using a meat grinder, Contherm™ scraped-surface heat exchanger, 2-phase decanter centrifuge and Westfalia polishing centrifuge. The extracted oils were stabilized using a food grade industrial antioxidant ('Dadex Toro', Caldic Canada Inc, Mississauga Ontario, Canada) immediately after extraction and no further oil refining was performed. All four crude oils were stored in a dark place at room temperature (18-20°C) in tightly sealed containers.

Physical properties

Smell, colour and physical state: Odour, colour and physical state of the oils were assessed by sensory evaluation.

Specific gravity: Specific gravity was determined using a hydrometer (Model # 11582, Fisher Scientific, Ottawa, Ontario, Canada) at 15.5°C (60°F).

Water content: The water content was determined using a water test kit (01-WTK-DELUXE, Sandy Brae Laboratories Inc., Wilmington, Delaware, USA) which measures the pressure produced after the reaction of trace water with calcium hydride in a pressure chamber [26].

Chemical properties

pH: The pH was measured using a pH pen (Model #850050, Super Scientific, Scottsdale, AZ, USA).

Ash content: The ash content was determined gravimetrically using a muffle furnace (Thermolyne Type F6000, Thermo Scientific, Asheville, North Carolina, USA) at 550°C following BS ISO 6884:2008 (British Standards Institution International Organization for Standardization). Successive portions of oil were ashed after initial ashing until a sufficient quantity was obtained to calculate the percent ash.

Saponification value: The saponification value was determined by following ASTM procedure D5558-95. To determine the saponification value, 4-5 g of oil sample was accurately weighed and filtered through a Whatman No. 40 filter paper to remove moisture and impurities. The oil samples after filtration were collected in a round bottom flask. An aliquot of 50 mL of alcoholic potassium hydroxide was added to the oil sample using a pipette and the pipette was allowed to drain for a definitive period of time. The alcoholic potassium hydroxide was prepared by adding a few grams (5 to 10 g) of potassium hydroxide to 1.5 L of 95% ethyl alcohol and boiled for 30-60 min under a reflex condenser on heating mantle. The boiled ethyl alcohol was distilled in

a rotary evaporator and collected. Potassium hydroxide (40 g) (low in carbonate) was dissolved in 1 L of distilled ethyl alcohol at 15.5°C until the solution was clear. A blank solution without oil was also prepared and the experiment was carried out simultaneously. The oil sample and the blank sample were gently boiled until the sample completely saponified. Complete saponification took place approximately in 1 h and it was generally determined by the clarity and homogeneity of the test solution. After 1 h, the samples were cooled down but not sufficiently to gel the content of the sample and the condenser was washed with a little amount of distilled water. To the samples, 1 mL of phenolphthalein indicator was added and titrated against 0.5 N HCl until the pink color disappeared completely. The saponification value of the oil sample was calculated by Equation 1 [96]

$$Saponification\,Value\,(mg\,KOH/g) = \frac{56.1 \times N(A-B)}{wt.of\ oil\,(\mathrm{g})} \quad (1)$$

N = Normality of HCl

A = Titration of blank (mL)

B = Titration of sample (mL)

56.1 = Molecular weight of potassium hydroxide (KOH)

Free fatty acids and Acid number: The free fatty acid content (%FFA) and Acid number (AN) were determined according to AOCS Official method Ca 5a-40 [97]. A well-mixed oil sample (7.05 ± 0.05 g) was accurately weighed into a 250 mL Erlenmeyer flask and 75 mL of hot neutralized 95% ethanol and 2 mL of 1% phenolphthalein indicator solution was then added to the oil sample. The hot neutralized 95% ethanol was prepared by heating 75 mL of 95% ethanol with 2 mL of 1% phenolphthalein indicator solution to incipient boiling and then ethanol was neutralized by adding 0.25N sodium hydroxide solution until a faint permanent pink color appeared. The oil samples were then titrated against 0.25N sodium hydroxide until the appearance of the first permanent pink color of the same intensity as that of the neutralized ethanol before the addition of sample. The permanent pink color must persist for at least 30 seconds during titration. The free fatty acids content (%FFA) and acid number were calculated using Equations 2 and 3.

$$FFA\,(\%) = \frac{mL\ of\ alkali \times N \times 28.2}{w} \quad (2)$$

Where:

N = Normality of NaOH solution

W = Weight of oil (g)

$$Acid\ number\ (mg\ KOH/g) = 1.99 \times FFA\ (\%) \quad (3)$$

Iodine value: The iodine value was determined by following AOAC official method 993.20. The test oil sample was filtered through a Whatman No. 40 filter paper to remove moisture and impurities. A 0.16 g sample of filtered fish oil was accurately weighed to 0.2 mg in a 250 mL Erlenmeyer flask. To the fish oil sample 15 mL of cyclohexane-acetic acid solvent was added and swirled to mix with a magnetic stirrer. Two blank solutions were also prepared simultaneously without the addition of fish oil. Wijs solution (25 mL) was added and the flask was then stored in a dry dark place for 2 h at 25 ± 5°C. After 2 h, the reaction was terminated by adding 20 mL of potassium iodide (KI) and and swirling to mix and then adding 150 mL of distilled water within 3 min. The samples were titrated against standardized 0.086 M (0.1M) standard sodium thiosulfate ($Na_2S_2O_3$) until the yellow colour of the solution has disappeared. A 1-2 mL of starch indicator was added to the samples and the titration was continued until the blue color of the solution disappeared. The iodine value of the oil sample was calculated using Equation 4 [27]:

$$Iodine\,Value = \frac{(\mathrm{B}-\mathrm{S}) \times M \times 12.96}{wt.of\ oil\,(\mathrm{g})} \quad (4)$$

Where:

B = Titration of blank (mL)

S = Titration of test solution (mL)

M = Molarity of $Na_2S_2O_3$

Peroxide value (PV): The peroxide value was determined by AOCS official method Cd 8-53. The test oil sample was filtered through a Whatman No. 40 filter paper to remove moisture and impurities. A 5 g sample of filtered oil was accurately weighed to 0.05 g in a 250 mL Erlenmeyer flask and 30 mL of 3:2 acetic acid-chloroform was added and swirled to mix well using a magnetic stirrer. Two blank samples were simultaneously prepared without addition of fish oil. To the samples, 0.5 mL of saturated potassium iodide solution was added and allowed to stand for 1 min. Saturated potassium iodide solution was prepared by adding 10 g potassium iodide to 6 mL boiled distilled water so that undissolved potassium iodide crystals were present during analysis. Then 30 mL of distilled water was added to the oil samples and swirled to mix with a magnetic stirrer. The samples were titrated against 0.1 N sodium thiosulfate until the yellow iodine color disappeared. Starch indicator (2 mL) was added and the titration was continued against 0.1 N sodium thiosulfate until the blue color disappeared. The blank titration value must not exceed 0.1 mL and the peroxide value was calculated by using Equation 5. Preliminary results showed a titration value less than 0.5 mL and therefore the peroxide value determination was carried out using 0.01 N sodium thiosulfate [28].

$$Peroxide\,Value\,(\mathrm{milliequivalents}/1000\,\mathrm{g\ sample}) = \frac{(\mathrm{S}-\mathrm{B}) \times N \times 1000}{W} \quad (5)$$

Where:

S = Volume of titrated sample (mL)

B = Volume of titrated blank (mL)

N = Normality of sodium thiosulfate solution

W = Weight of oil (g)

***p*-Anisidine value:** The *p*-anisidine value was determined by AOCS official method Cd 18-90. The test oil sample was filtered through a Whatman No. 40 filter paper to remove moisture and impurities. A 0.5-4 ± 0.001g sample of oil was accurately weighed in a 25 mL volumetric flask. The oil samples were dissolved and diluted with 25 mL iso-octane. The absorbance (A_B) of the oil sample was measured at 350 nm using a spectrophotometer (Jenway 6400/6405, Jenway Incorporated, Stone, Staffordshire, UK). A 5 mL sample of oil was pipetted into one test tube and 1 mL of *p*-anisidine reagent was added. A 5 mL of iso-octane was added to another test tube and 1 mL of p-anisidine reagent was added to it and used as a blank. The p-anisidine reagent was prepared by adding 0.25 g p-anisidine to 100 mL of glacial acetic acid. After 10 minutes, the absorbance (A_S) of the oil sample with the p-anisidine reagent was measured at 350 nm using a spectrophotometer. The *p*-anisidine value was calculated by using following Equation 6 [29].

$$p-anisidine\,value = \frac{25 \times (1.2\,\mathrm{A_S} - \mathrm{A_B})}{W} \quad (6)$$

Where:

A_S = Absorbance of the fat solution after reaction with

the *p*-anisidine reagent

A_B = Absorbance of the fat solution

W = Weight of oil (g)

TOTOX value: TOTOX means "Total Oxidation", calculated as twice the Peroxide value plus Anisidine value.

Flash point: The flash point was measured using a Pensky-Marten closed cup tester (K162XX, Koehler Instruments, Bohemia, New York, USA) [98] according to Procedure A in ASTM D93-12. The test cup was filled with 75 mL of oil sample and the cup was closed with a test cover and placed in the assembly, ensuring that the locating groove was engaged. The temperature of the test cup and test specimen should be at least 18°C below the expected flash point. The test flame was switched on and the oil was heated at a rate of 5-6°C/minute. The oil sample was stirred in a downward direction at 90-120 rpm. The observed flash point was recorded at the temperature when a distinct flash occurred in the interior of the cup. The oil sample was deemed to have flashed when a large flame appeared and instantaneously propagated itself over the entire surface of the oil sample. The observed flash point was corrected for barometric pressure.

Viscosity: Dynamic viscosity (cP) was measured with a Brookfield viscometer (DV-I, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a S61 spindle at 50 rpm at 40°C. Kinematic viscosity (cSt) was calculated using equation 7:

$$v = \frac{\mu}{\rho} \quad (7)$$

Where:

v = Kinematic viscosity (mm^2/s)

μ = Dynamic viscosity (N_s/m^2)

ρ = Density (kg/m^3)

Refractive index: The refractive index was measured with a pocket ATAGO refractometer (PAL-S, ATAGO Co. Ltd., Tokyo, Japan) per manufacturer's instructions. 2-3 drops of the crude oil was applied to the prism surface. The start key was pressed and the BRIX value was displayed and recorded. The Brix value was converted to refractive index.

Cetane number: The Cetane Number was calculated using equation (8) [30].

$$CN = 46.3 + \frac{5458}{SN} - .225 \times IV \quad (8)$$

Where:

CN = Cetane number

SN = Saponification number of oil (mg KOH/g)

IV = Iodine value of oil

Higher heating value: The higher heating value (HHV) was calculated using equation (9) [31].

$$HHV = 0.0317VS + 38.053 \quad (9)$$

Where:

HHV = Higher Heating Value (kJ/g)

VS = Kinematic Viscosity (mm^2/s) .

Lipid class determination and fatty acid analysis

Lipid extraction: Lipid samples were extracted according to Parrish [32]. An aliquot of 250 µl of oil (between 170 and 215 mg) sample was weighed in a test tube containing 2 mL of chloroform. Previous to addition of the oil sample, the test tubes and Teflon® lined caps were rinsed 3 times with methanol and chloroform, respectively. 1 mL of ice-cold methanol, 1 mL of 2:1 chloroform:methanol and 0.5 mL of chloroform extracted water were added to the test tube. Chloroform extracted water was prepared by adding 1L of distilled water and 30 mL of chloroform to a separating funnel. The funnel was manually shaken for 2 minutes and the chloroform was allowed to settle and removed from the bottom of the funnel. This procedure was repeated twice to remove any lipids present in the distilled water. The test tube was then recapped and sonicated for 10 minutes followed by centrifugation for 2-3 minutes at 3000 rpm using an international clinical centrifuge (model CL, International Equipment Co, Needham, Mass). The entire lower organic lipid layer was removed by a double pipetting technique and transferred to a 15 mL vial that was cleaned 3 times with methanol and chloroform, respectively [99]. The double pipetting technique was performed in three steps. Firstly, an ashed 14 cm pipette was passed through the top aqueous layer in the test tube, by bubbling air with the pipette bulb to prevent the aqueous layer from entering the 14 cm pipette until it touched the bottom of the test tube. Secondly, the pipette bulb was removed and a 27 cm pipette was placed inside the shorter pipette until it touched the bottom of the test tube. Thirdly, the lipid layer was removed using the long pipette and transferred to a second cleaned 15 mL vial. Each of the short and long pipettes were washed with 3 mL ice-cold chloroform and the wash was collected, subsequently. The samples were again resonicated, centrifuged, double pipetted and the pipettes were rinsed three times as previously described and all the organic layers were pooled together. The extracted lipid was then evaporated under a gentle stream of nitrogen, sealed with Teflon® tape and stored in the freezer at -20°C until use.

Lipid class composition: Lipid class composition was determined using an Iatroscan Mark VI TLC-FID (Iatron Laboratories Inc., Tokyo, Japan), silica coated Chromarods and a three-step development method [33]. The Chromarods were calibrated (0.2–20 µg) using lipid standards including: n-nonadecane (HC-aliphatic hydrocarbon), cholesteryl palmitate (WE/SE-wax esters/steryl ester), n-hexdecan-3-one (KET-ketone), glyceryl tripalmitate (TAG-triacylglycerol), glyceryl-1,2-dihexadecanoate (DG-diglyceride), 1-hexadecanol (ALC-free aliphatic alcohol), cholesterol (ST-free sterol), 1-monopalmitoyl-rac-glycerol (AMPL-acetone mobile phase lipids) and 1,2, di-0-hexadecyl-sn-glycerol-3-phosphatidylcholine (PL-phospholipids) that were obtained from Sigma–Aldrich from Sigma Chemicals (Sigma Chemicals, St. Louis, Mo., USA).

The lipid extracts and standards were applied to the Chromarods and focused to a narrow band using 100% acetone. During standard or sample application, the frame holding the chromarods were placed on a warm hot-plate with the lower edge extending beyond the end of the hot plate so that the bottom of the rods were not directly over the heat source.

Four different solvent systems were used to obtain three chromatograms per rod. The first development system was hexane: diethyl ether: formic acid (99.95:1:0.05). The rods were developed for 25 minutes, removed from the system for 5 minutes and placed again in the system for 20 minutes for double development. The first chromatograms were obtained by scanning each rod to the lowest point behind the ketone (KET) peak. The second development was for

40 minutes in hexane:diethyl ether:formic acid (79:20:1). The second chromatogram was obtained by scanning each rod to the lowest point behind the diglyceride (DG) peak. The final development was carried out in two steps, in the first step the lipid extracted was developed using 100% acetone for two 15 minute time periods, followed by two 10 minute periods in chloroform:methanol:chloroform-extracted water (5:4:1). The third chromatogram was obtained as the complete scan after two double developments. Before each solvent system the rods were dried in a constant humidity chamber. After each development system the rods were scanned in the Iatroscan and the data collected using Peak Simple software (ver 3.67, SRI Inc).

Preparation of fatty acid methyl esters (FAME) with H_2SO_4 in MeOH: An aliquot of 40 µl of lipid extract was transferred to a lipid cleaned (rinsed 3 times with methanol and chloroform, respectively) vial and 1.5 mL of methylene chloride and 3.0 mL Hilditch reagent were added, subsequently. The Hilditch reagent was prepared by adding 1.5 mL of concentrated H_2SO_4 to 100 mL of dry methanol (100 mL methanol was transferred to a volumetric flask and sufficient amount of Na_2SO_4 was added to the methanol to cover the bottom of the flask and mixed manually by inverting the flask and left for 10 minutes and then decanted). The sample was capped and vortexed for approximately 5 seconds followed by sonication for 4 minutes. The tube was then flushed with nitrogen, capped, sealed with Teflon® tape and heated at 100°C for 1 hour in a VWR drying oven (VWR international, Mississauga, Ontario, Canada). The vials were then cooled to room temperature. Approximately 0.5 mL of saturated sodium bicarbonate solution (9 g/100 mL of chloroform extracted water) was slowly and carefully added to the vial, followed by addition of 1.5 mL of hexane and vortexing for 5-10 seconds. The top organic layer was carefully removed to a new vial without disturbing the bottom layer and the hexane was evaporated with a gentle stream of nitrogen. The fatty acids were re-suspended by adding approximately 0.5 mL of hexane, capping the vial with nitrogen, and Teflon® tape and sonicating for an additional 4 minutes.

FAME analysis: An aliquot of 10 µL of the mixture was separated by fatty acid class based on the carbon atom by a gas chromatography system (HP6890 Series II, Agilent Technologies, Mississauga, Ontario, Canada), coupled with flame ionization detector (FID) and 7683 auto sampler. A ZB wax + polar capillary column 30 m in length, 0.32 mm of internal diameter and 0.25 µm film thicknesses (Phenomenex, Torrance, CA, USA) was used for analyses. The separated samples were injected directly into the column with the initial oven temperature of 65°C for 5 minutes, followed by ramped temperature of 195°C at a rate of 40°C/min for 15 minutes and again ramped to a final temperature of 220°C at a rate of 2°C/min. A final temperature of 220°C was held for 0.75 minutes. The detection system was equipped with a flame ionization detector (FID) operating at 260°C with hydrogen as a carrier gas at a flow rate of 2 mL/min. The injector temperature was started at 150°C and ramped to a final temperature of 250°C at a rate of 120°C/minute. Peaks were identified using retention times from standards purchased from Supelco: 37 component FAME mix (Product number 47885-U), Bacterial acid methyl ester mix (product number 47080-U), PUFA 1 (product number 47033) and PUFA 3 (product number 47085-U). Chromatograms were integrated using the Varian Galaxie Chromatography Data System, version 1.9.3.2. The total run time was 32 minutes.

Results and Discussion

Physical properties

Physical properties of four crude marine oils including farmed salmon, seal, cod liver and wild salmon are shown in Table 1.

Smell, colour and physical state: All four marine oils had an agreeably oily smell while cod liver and wild salmon also had a slightly fishy smell. Seal and cod liver oils were pale yellow in color while farmed Atlantic salmon and wild Pacific salmon had an orange and light orange colours, respectively. The crude marine oils were in a clear liquid state at room temperature except wild pacific salmon oil which was a cloudy liquid with small solid particles in suspension at room temperature.

Specific gravity: Specific gravity is the ratio of the density of the substance to that of water (1 g/cm^3) at 15.6°C. It can be used to determine the purity of oil to match desired standards. Specific gravity of farmed salmon, seal, cod liver and wild salmon oil was 0.921, 0.923, 0.924 and 0.922 g/cm^3, respectively, which were close to the biodiesel standard range of 0.87–0.90 g/cm^3 [34,35]. The higher viscosity of oils tends to cause problems including incomplete combustion and particulate matter emissions when used directly in diesel engines [36,37]. Trans-esterification of oils and fats using short-chain alcohols result in monoesters having viscosities closer to petroleum- based diesel fuel [38,39]. The specific gravity of biodiesel will depend on the type of feed stock, fatty acid composition of the mixed esters and their purity. It is very essential to monitor specific gravity of diesel fuel and correlate against performance indicators, such as cetane number and heating value. However, the specific gravities of hydrocarbons are strongly affected by temperature [40].

Water content: The water content of farmed salmon, seal, cod liver and wild salmon oil was 325, 689, 179 and 312 ppm, respectively. Obtained water content values were within the ASTM biodiesel standards (<500 ppm) except for seal oil. The water content in oil must be within ASTM standards to prevent excessive soap formation during the chemical trans-esterification reaction. The produced soap also increases the viscosity of the reaction mixture, sometimes causing gel formation which can traps the resulting ester and glycerin together thereby making the separation of glycerol from ester difficult [41]. Most vegetable and marine oils are degummed in an extraction plant using

S.No	Properties	Crude Farmed Salmon Oil (Atlantic)	Crude Seal Oil	Crude Cod Liver Oil	Crude Wild Salmon Oil (Pacific)	ASTM specification for Biodiesel
1	Odour	Fresh oil odour, not fishy	Fresh oil odour, not fishy	Fresh oil odour, slightly fishy	Fresh oil odour, slightly fishy	---
2	Color	Orange	Pale yellow	Pale yellow	Pale orange	---
3	Specific gravity	0.921	0.923	0.924	0.922	---
4	Water content (ppm)	325	689	179	312	< 500
5	Physical state at room temperature	Clear liquid	Clear liquid	Clear liquid	Cloudy liquid, small solid particles suspended in otherwise clear oil.	---

Table 1: Physical properties of four crude marine oils.

low amounts of water and the used water is removed subsequently during centrifugation. Most vegetable oils have a clear appearance even though they hold 200-1000 ppm water in the dissolved state. Cloudy or hazy oil/FAME indicates water above 1500 ppm (0.15%) at room temperature. Generally it is recommended that feedstock oil should not contain more than 1500 ppm of water to ensure successful trans-esterification. If it does, a drying step has to be conducted prior to trans-esterification. Free water can be removed by centrifugation or settling out at elevated temperature. Dissolved water in contrast has to be removed by steaming off (i.e. heating to near the boiling point of water) [42,43]. The higher water content in biodiesel can cause corrosion of internal combustion engine components such as pumps, injectors and fuel line tubes and affect heat of combustion which results in greater power consumption [44]. The presence of higher amounts of water also allows microbes to grow during storage and may affect the engine components such as filters and pumps. Formation of gelling and nucleation of oil/biodiesel can take place as water may freeze at low temperatures [45,46].

Chemical properties

Chemical properties of the four crude marine oils including farmed salmon, seal, cod liver and wild salmon are shown in Table 2.

pH: The reported pH values for all four marine oils were neutral in the range of 6.5-6.8. The pH of feedstock oil is a major factor in both base/acid catalyzed and enzyme catalyzed trans-esterification reaction, because the catalyst activity may be severely affected at lower or higher pH values. Devanesan et al., [47] studied the effect of pH while producing biodiesel from Jatropha oil using immobilized *Pseudomonas fluorescence* and reported an optimum pH value of 7 for biodiesel production. Yee et al., [48] reported that the activity and stability of the enzyme during enzymatic trans-esterification depends upon the pH of the reaction mixture. Different enzymes have different pH ranges and the optimal pH range not only depends on the nature of the enzyme but also on the substrate, substrate concentration and stability of the enzyme. Kulkarni and Pandit [49] reported an optimum pH of 7.0 for enzymatic trans-esterification of castor oil for biodiesel production.

Ash content: Ash consists of the residue left after the fuel is heated to a sufficiently high temperature when combustible material burns and leaves as CO_2 and H_2O. The ash content of farmed salmon, seal, cod liver and wild salmon oil was 0.0045, 0.0027, 0.0037 and 0.0034%, respectively. The ash content of all four marine oils was much lower than the ASTM biodiesel standards (0.02%). Contaminants including abrasive solids, soluble metallic soaps, inorganic materials and unremoved catalyst present in the fuel may produce ash during combustion that can be abrasive and contribute to wear in fuel injector, fuel pump, piston and ring wear. Sodium and potassium metals are likely to be the main sources for ash in biodiesel. Higher amounts of ash content in the feedstock oil tends to produce rejected quality of biodiesel according to ASTM standards [50,51].

Saponification value: When oil and fat reacts with alkali their long chain fatty acid salts results in soap formation, glycerols and fatty acids. Soaps, which are the salts of longer chain fatty acids, are produced by treating a fat with alkali [52,53]. The saponification value, which is defined as the number of milligrams of potassium hydroxide required to saponify 1 g of fat, is an indicator of the average molecular weight of the triacylglycerols into the fat sample. Dividing the mean molecular weight by 3 gives an approximate mean molecular weight for the fatty acids present in the fat sample [52]. Fat that is composed of short chain fatty acids will have a greater number of fatty acids compared to fats containing long chain fatty acids [54]. Boran et al., [24] studied the effect of temperature and time on saponification value of garfish, golden mullet, shad and horse mackerel oil. The fish oils were stored at 4 and -18°C prior to the analysis. The results from this study indicated that the saponification value of all the fish oils increased at both temperatures. The highest increase in the saponification value was found in fish oil stored at 4°C. During the storage of fish oil, hydrolysis and oxidation causes lipid breakdown forming free fatty acids, aldehydes and ketones. These end products can contributes to an increase in saponification value. The results also indicated that the saponification values of garfish, golden mullet, shad and horse mackerel oil were in the range of 181-195, 176-187, 191-200 and 192-202 mg KOH/g, respectively. Fish oils stored at -18°C had a lower saponification value and twice the longer shelf life than samples stored at 4°C. Garfish and shad oil showed the greatest stability against oxidation and preserved their acceptability properties for at least 150 days at -18°C. In the present study, the saponification value of farmed salmon, seal, cod liver and wild salmon oil was 185.85, 184.61, 179.55, 176.19 mg KOH/g, respectively. These

S. No	Properties	Crude Farmed Salmon Oil (Atlantic)	Crude Seal Oil	Crude Cod liver Oil	Crude Wild Salmon Oil (Pacific)
1	pH	6.8	6.5	6.8	6.7
2	Ash content (%)	0.0045	0.0027	0.0037	0.0034
3	Acid value (mg KOH/g)	2.441	0.958	0.057	0.771
4	Iodine value	116.79	138.67	139.15	138.79
5	Saponification value (mg KOH/g)	185.85	184.61	179.55	176.19
6	p-Anisidine value	3.36	4.21	6.20	9.67
7	Peroxide value (meq/kg)	9.17	7.04	6.92	5.13
8	TOTOX Value	21.69	18.29	20.03	19.92
9	Free fatty acid (%)	1.23	0.48	0.03	0.39
10	Flash point (°C)	267.5	284.0	251.0	232.0
11	Kinematic viscosity (mm^2/s, 40°C)	29.35	26.08	27.46	26.79
12	Refractive index	1.47	1.48	1.48	1.48
13	Cetane number	49.39	44.67	45.39	46.05
14	Higher heating value (kJ/g)	39.00	38.88	38.92	38.90

Table 2: Chemical properties.

values were within the standard range of 176-195 [55]. This indicates a high proportion of fatty acids of low molecular weight. This shows a low tendency towards soap formation and less difficulties in the separation of products if utilized as feedstock for biodiesel production.

Free fatty acids: It is important to determine free fatty acids (FFA) content as it is one of the main criteria for the quality determination of fats and oils. Also, FFA was employed to assess fish deterioration during frozen storage [56] and FFA content increases with increase in storage time. According to Miyashita and Takagi [57], FFA have shown a catalytic effect on lipid oxidation development on soybean, safflower, olive and linseed lipids. Higher amounts of FFA in feedstock oils limits their use for biodiesel production by acid-catalyzed and alkali-catalyzed methods. In the case of biodiesel production by the alkali-catalyzed method, the maximum allowable is below 2.5 wt.% FFA. A pretreatment step becomes necessary if the oil or fat feedstock has a FFA content over 2.5 wt.% [58]. Aryee et al., [59] reported that lipids extracted from fish tissue are highly prone to both lipolysis and oxidation. The presence of more than 0.5% FFA in the oil can cause soap formation and reduce the efficiency of the catalyst during trans-esterification. The author studied free fatty acid formation in stored crude fish oil at -20°C over a period of 120 days. In this study, the free fatty acids (FFA) content in all four oils were relatively low compared to allowable limits and indicates that esterification prior to trans-esterification may not be necessary and a one-step reaction can complete the trans-esterification process and result in a higher yield of biodiesel. However, Math et al., [60] reported that the most effective conversion of triglycerides into esters during the alkaline catalyzed trans-esterification process can take place when the free fatty acid level is less than 1%. The acid value (AV) is approximately 2 mg KOH/g oil at 1% FFA concentration.

Acid value: Oil acidity is an important quality parameter determining the presence of free fatty acid (FFA) and other non-lipid acid compounds [61]. FFA are mostly generated by a hydrolysis reaction of triacylglycerides. Oil acidity depends on several factors such as oil composition, extraction procedures and raw material freshness. The acid value quantifies the amount of acid present in the sample. It is the mass of potassium hydroxide in mg that is required to neutralize 1g of chemical substance [23]. As oil goes rancid, triacylglyceride (TAG) coverts to fatty acid (FA) and glycerol which increases the acid number. Kai et al., [62] reported that the acid value in feedstock oil should be less than 2.5 mg KOH/g oil for the base-catalyzed transesterification process. However, Ding et al., [63] reported that acid value of oil should be less than 1 mg KOH/g oil to meet the alkaline catalyzed trans-esterification conditions. The recommended ASTM standard for acid value of biodiesel is 0.8 mg KOH/g [58]. Freedman et al., [64] obtained maximum ester yield using moisture free alcohol and vegetable oil with an acid value less than 1 mg KOH/g. In the present study, the acid value of farmed salmon, seal, cod liver and wild salmon oil were 2.441, 0.958, 0.057 and 0.771 mg KOH/g, respectively. The acid values of cod liver and wild salmon oils were below the ASTM biodiesel standards (0.8 mg KOH/g) except for farmed salmon and seal oil.

Iodine value: The iodine number gives an indication about the amount of unsaturated fatty compounds (number of double bonds) in the oil and thereby indicates the ease of oxidation or drying capacity of the oil. However, it does not give any information on the nature of the unsaturated and saturated compounds [50]. As the number of double bonds does not change during trans-esterification, measuring the iodine value in feedstock oil will give an indication of the stability of the biodiesel produced from the same feedstock. Europe's EN14214 specification allows a maximum of 120 for the Iodine number (g I_2/100 g) in biodiesel [65]. In this present study, the iodine value of farmed salmon, seal, cod liver and wild salmon oil were 116.79, 138.67, 139.15 and 138.79 g I_2/100 g, respectively. Oils are classified as drying, semi drying and non-drying on the basis of iodine value. Oils with an iodine value above 125 g I_2/100 g are classified as drying oils; those with an iodine value 110–140 g I_2/100 g are classified as semidrying oils and those with iodine value less than 110 g I_2/100 g are considered as non-drying oils [34]. All the characterised marine oils were drying oils except farmed salmon oil which was a semidrying oil. Drying oils are susceptible to become rancid and cannot be preserved for long without the addition of an antioxidant. They can cause the lowering of the pour point of biodiesel produced. All four marine oils were more likely to polymerize in the heat of the engine if used directly without trans-esterification [66].

Peroxide value (PV): The peroxide value is the measurement of the primary oxidation product hydroperoxide and is a widely used chemical test for the determination of fats and oil quality [67]. The oxidative process of oils and fats is one of the main causes of the deterioration of the principal organoleptic and nutritional characteristics of foodstuffs. The number of peroxides present in the oil is an index of their primary oxidative level and its tendency to go rancid. The complex oxidation process can be summarized into two phases: in the first phase, fatty acids react with oxygen and determine odorless compounds as peroxides; during the second phase the peroxides degrade into many substances as volatile aldehydes, responsible for the rancid odor and flavor and in the non-volatile portion. The lower the peroxide value the better the oil quality and its state of preservation [25]. In the present study, the peroxide values of farmed salmon, seal, cod liver and wild salmon oil were 9.17, 7.04, 6.92 and 5.13 meq O_2/kg oil, respectively. Bimbo [68] reported acceptable PV values for fish oil were between 3 and 20. In this study, PVs of all examined fish oil samples did not exceed 20 meq O_2/kg oil. However, according to Boran [24], the acceptability limit for PV of crude fish oil is 7–8 meq O_2/kg oil and all examined oil samples did not exceed this limit and showed oxidative stability except farmed salmon oil. If peroxide values are higher, oils will not remain stable and become rancid easily and should be utilized immediately after extraction. Fritsche and Johnston [69] reported that peroxide value of the purified diet containing menhaden oil without antioxidant had elevated 5-6 folds within 24 h and 12 fold within 48 h when exposed to air at room temperature.

***p*-Anisidine value (AV):** The *p*-anisidine value is used to measure the secondary products of oxidation and determines the aldehyde in the lipid, primarily 2-alkene present in the fat. Aldehyde present in the oil and the p-anisidine reagent react under acidic condition [25,100]. The color obtained not only depends on the aldehydes present, but also their structure. Further degradation of lipids generates off-flavours and off-odours. On the contrary, other tests consider the volatile portion of aldehydes and, due to their intrinsic variable nature, result in less reliable data [22]. In the present study, the *p*-Anisidine values of farmed salmon, seal, cod liver and wild salmon oil were 3.36, 4.21, 6.20 and 9.67, respectively. All studied marine oils *p*-Anisidine values are within the recommended range of ≤20 for crude fish oil [71]. Pak [72] studied the stability and quality of fish oil and found the p-anisidine value to be 19.8. Che Man and Setiowaty [73] studied the *p*-anisidine value of oxidized palm olein using FTIR and reported the p-anisidine value of 8.684.

The formation of primary and secondary degradation products can be prevented by adding a stabilizing agent (antioxidant) immediately

after oil extraction. However, it is very much essential to perform peroxide and p-anisidine value tests prior to the alkaline catalyzed trans-esterification process for biodiesel production.

Totox value: The p-anisidine value is often used in conjunction with the peroxide value to calculate the total oxidation value or Totox value. The Totox value is calculated by the formula AV + 2PV to determine the overall oxidation state of the oil. The peroxide value can decrease over time so p-anisidine value and/or Totox calculation are essential to get an understanding of total oxidation. FAO and WHO [71] recommended Totox value is ≤ 26 for fish oil. In the present study, Totox values of all the examined four marine oils were within the recommended range.

Flash point: The flash point is the lowest temperature at which a product of petroleum gives off sufficient flammable vapours to ignite or momentarily flash. The value of the flash point is used for the classification of flammable and combustible materials needed for safety and shipping regulations. The flash point is determined by heating a sample of the fuel in a stirred container and passing a flame over the surface of the liquid. If the temperature is at or above the flash point, the vapor will ignite and an easily detectable flash can be observed [74]. In this study, the flash points of farmed salmon, seal, cod liver and wild salmon oils were 267.5, 284.0, 251.0 and 232.0°C, respectively. The flash point of all four marine oil samples were above the recommended ASTM D6751 value of 130°C and therefore pose no risk of fire outbreaks in case of accidents. They were all also above the 120°C minimum EN 14214 (European) recommended range. It is very essential to measure the alcohol content and flash point after each batch of biodiesel produced as very small quantities of residual alcohol present in biodiesel can significantly decrease the flash point [75].

Kinematic viscosity [cSt]: Viscosity is a measure of a fluid's resistance to flow. The greater the viscosity, the less readily the liquid flows. It is one of the most important parameters required in the design of combustion processes. A direct relationship exists between the oil viscosity and some chemical characteristics of the lipids including the degree of unsaturation and chain length of the fatty acids that constitute the triacylglycerols. Viscosity slightly decreases with increased degree of unsaturation and rapidly increases with polymerisation [76,77]. Abromovič and Klofutar [76] reported that viscosity linearly decreases as the iodine value increases and there was no dependence of viscosity on saponification value, which characterises the chain length of fatty acids. Gunstone [78] reported that the viscosity of oil is highly temperature dependent and each oil has a specific viscosity curve, which depends on its chemical composition and presence and quantity of impurities. Dubey et al., [79] found the reduction (7.4 times) in viscosity while the temperature was raised from 10-70°C and reported a viscosity of 47.3cSt at 20°C. In the present study, all crude marine oils showed a viscosity between 26-30 cSt at 40°C. The viscosity of biodiesel is highly dependent on the type of feedstock oil. The range for biodiesel is between 1.9 and 6.0 cSt as specified by ASTM standard D D6751-12. The major concern about biodiesel is that its viscosity tends to be higher than diesel fuel. Biodiesel consisting of the methyl esters of soybean oil has a viscosity between 4.2 and 4.6 cSt [80] while ethyl esters of rapeseed oil, have been reported to be as high as 6.0 cSt [81].

Refractive index: The refractive index is used to measure the increase in autoxidation of fats and oils. The refractive index of oils depends on their molecular weight, fatty acid chain length, degree of unsaturation, and degree of conjugation. The refractive index of an oil increases (nonlinearly) with chain length and unsaturation of fatty acids. The refractive indices and peroxide values of the oils and fats can significantly increase while exposed to light and heat [82]. Generally oils' refractive index values vary between 1.447 and 1.482. The degree of unsaturation of an oil greatly influences its oxidative stability and the stability of the biodiesel derived from it [83]. The fatty acid analysis shows a high degree of unsaturation in all four marine oils tested in this study. Hence, the stabilization of the oil as well as the biodiesel immediately after extraction/production is crucial for obtaining a high quality biofuel. Sadrolhosseini et al., [84] reported that the state of biodiesel can be evaluated based on change of refractive index. Biodiesel turns into cloudy state when the biodiesel temperature is near to the cloud point. In this study, refractive index of farmed salmon, seal, cod liver and wild salmon oils were 1.47, 1.48, 1.48 and 1.48, respectively.

Cetane number: The cetane number is used to measure the combustion quality of biodiesel and diesel fuels during compression ignition. In this study, the calculated cetane numbers of farmed salmon, seal, cod liver and wild salmon oils were 49.39, 44.67, 45.39 and 46.05, respectively. Except for farmed salmon oil, these values are lower than the ASTM D6751-12 recommended cetane number minimum of 47. Bello et al., [85] found the cetane number of cashew nut, egusi melon and rubber seed oils increased 10-40% after trans-esterification and the increase depended on the degree of unsaturation. Longer fatty acid carbon chains and more saturated molecules leads to higher cetane numbers [86]. It is expected that trans-esterification of the studied crude marine oils would result in an increase in the cetane numbers.

Higher heating value: Higher Heating Value is an important property defining the thermal energy released per unit quantity of fuel after complete combustion of fuel to CO_2 and H_2O at the initial temperature and pressure. It is an important property that describes the energy content of fuels, such as oils and biodiesel. HHV can be determined by use of a bomb calorimeter or calculated from fuel characteristics and physical properties. Demirbas [31] found high regression between viscosity and HHV of vegetable oil and biodiesel and suggested that the correlations may be used to estimate the HHV of mixtures of biodiesels obtained from vegetable oils. Sardine oil has a HHV of 39.6, whereas, sardine oil biodiesel has a HHV of 39.7 [87]. In this study, the calculated higher heating values of farmed salmon, seal, cod liver and wild salmon oils were 39.00, 38.88, 38.92 and 38.90, respectively. These values are lower than the ASTM D6751 recommended higher heating value minimum of 39.2.

Lipid classification

Lipid classes of the farmed salmon, seal, cod liver and wild salmon oil are presented in Table 3. Among the lipid classes, triacylglycerols, free fatty acids, phospholipids, sterols and polar lipids were the predominant constituents, while hydrocarbons were minor components in all the marine oils studied. Hydrocarbons, sterol esters and triacylglycerol were the major components of the neutral lipids in the four marine oils. The presence of a higher percentage (≥ 80%) of triacylglycerols was found in all four marine oils including farmed salmon (92.83%), seal (93%), cod liver (85.26%) and wild salmon (81.20%); which is essential for the trans-esterification of feedstock oil and higher biodiesel production yield. Hydrocarbon content was absent in the farmed and wild salmon oil, while 0.11 and 0.33% was present in the crude seal and cod liver oil, respectively. Farmed salmon, seal, cod liver and wild salmon oil also contained 3.74, 2.33, 3.24 and 2.02% of sterol esters, respectively. Cod liver (14.72%) and wild salmon oil (9.94%) were rich in polar lipids while the farmed salmon (2.43%) and wild salmon (2.44%) were low in polar lipids. The phospholipids present in the farmed salmon, seal, cod liver and wild salmon oil were 1.43, 1.53,

S. NO.	Parameter	Crude Farmed Salmon Oil (Atlantic)	Crude Seal Oil	Crude Cod liver Oil	Crude Wild Salmon Oil (Pacific)
1	Hydrocarbons	0.00	0.11	0.33	0.00
2	Steryl Esters/Wax Esters	0.00	0.12	0.00	0.00
3	Ethyl Esters	0.00	0.00	0.00	0.00
4	Methyl Esters	0.00	0.00	0.00	0.00
5	Ethyl Ketones	0.00	0.00	0.00	0.00
6	Methyl Ketones	0.00	0.00	0.00	0.00
7	Glycerol Ethers	0.00	0.00	0.00	0.00
8	Triacylglycerols	92.83	93	85.26	81.20
9	Free Fatty Acids	1.23	0.48	0.03	0.39 permanent pink
10	Alcohols	0.00	0.00	0.00	0.00
11	Sterols	3.74	2.33	3.24	2.02
12	Diacylglycerols	0.00	0.00	0.00	0.00
13	Acetone Mobile Polar Lipids	2.43	9.94	14.72	2.44
14	Phospholipids	1.43	1.51	1.23	1.67

Table 3: Composition (wt%) of marine oils lipid classes.

1.21 and 1.67%, respectively which are higher than the ASTM D6751-12 recommended limit of ≤10 ppm. Phospholipids (gums) in oils refer to hydratable and non-hydratable phosphatides, lecithin, sugars, trace metals and other impurities [88,89]. For biodiesel producers, gums are a concern for the following reasons: (a) inhibit the catalyst during the trans-esterification reaction (b) difficult to separate biodiesel and glycerol due to the emulsifying effect that occurs after the trans-esterification reaction (c) metals contamination in the final product (P and Ca specifically), and (d) yield loss. [43,64,90]. In the present study, all the characterized marine oils contain higher amounts of phospholipids and will require a degumming process prior to biodiesel production. Liu et al., [91] studied the removal of phospholipids from Jatropha oil through a conventional degumming process combined with ultrafiltration membrane separation in a small-scale batch system and reported that the phospholipid content of jatropha oil was reduced from 1200 ppm to 60 ppm and was further reduced to less than 20 ppm by subjecting the oil to ultrafiltration membrane separation. Fan et al., [43] reported a 99.7% reduction of phosphorus content in the crude soybean oil after acid and water degumming. A relatively low free fatty acid content in all four marine oils was observed compared to allowable limits of <2.5%. Math et al., [60] reported that the most effective conversion of triglycerides into esters during alkaline catalyzed trans-esterification process can take place when the free fatty acid level is less than 1% which indicates that esterification prior to trans-esterification may be necessary for Atlantic farmed salmon oil. El-Mashad et al., [92] concluded that only alkaline-catalysed trans-esterification was not an effective method for producing biodiesel from salmon oil and implemented a two-step process including a sulphuric acid-catalysed pretreatment followed by alkaline-catalysed transesterification and observed a reduction of the acid value from 12.0 to 3 mg KOH/g oil in the first step of acid catalysed pretreatment.

Fatty acid composition

There were about 78 fatty acids identified in each examined marine oil using the gas chromatography analysis however, only 12 fatty acids had values >0.5%. Fatty acid composition including saturated, monounsaturated and polyunsaturated fatty acids of marine oils are shown in Table 4. Farmed salmon, seal, cod liver and wild salmon oil have consisted of 19.83, 13.53, 16.05, and 22.52% of saturated fatty acids including myristic acid (14:0), palmitic Acid (16:0), stearic acid (18:0), respectively. Farmed salmon, seal, cod liver and wild salmon oil also contains 47.38, 58.88, 53.79 and 44.97% of monounsaturated fatty acids including palmitoleic acid (16:1n-7), oleic acid (18:1n-9), vaccenic acid (18:1n-7), respectively. The total polyunsaturated fatty acids including linoleic acid (18:2n-6), alpha-linolenic acid (18:3n-3), arachidonic (20:4n-6), EPA (20:5n-3), DPA (22:5n-3) and DHA (22:6n-3) were also present in the farmed salmon, seal, cod liver and wild salmon oil comprising of 32.23, 26.64, 29.05 and 31.42%, respectively. Biodiesel produced from saturated fat have a higher cetane number and improve oxidative stability however, they also have higher cloud point and poor temperature properties which can lead to gel formation at ambient temperature. If more polyunsaturated fatty acids are present in the feedstock oil then a reduced cloud point, cetane number and stability can be observed in the biodiesel. In the present study, all four studied marine oils have higher polyunsaturated fatty acids so biodiesel produced will have less oxidative stability and may results in the precipitation of biodiesel components in a fuel feeding system or combustion chamber [93-95]

Conclusion

In the present study, farmed salmon, seal, cod liver and wild salmon marine oils were characterized to identify their physical and chemical properties and determine their feasibility as a feedstock for biodiesel production. All oils were pale yellow to orange in color and were stable in the liquid state at room temperature. The specific gravity of all oils was significantly closer to the recommended parameters. The water content of all oils were within the recommended limit (<500 ppm) except for seal oil (689 ppm). A drying step has to be implemented to remove the water present in the feedstock seal oil as water in biodiesel can cause corrosion of internal combustion engine components and results in greater power consumption. The pH and ash content values of all oils were in the recommended range. All oils studied were comprised mainly of short chain fatty acids due to their high saponification values. The free fatty acids (FFA) of all oils were relatively low compared to the allowable limits of 2.5%. The acid values of all oils were within the ASTM biodiesel standards (0.8 mg KOH/g) except for farmed salmon and seal oil. All the characterised marine oils were identified as drying oils except farmed salmon oil which was a semidrying oil because of the higher iodine value (116.79-139.15 g I2/100 g) and are susceptible to become rancid which causes reduction of pour point of biodiesel produced in the absence of antioxidant. All four marine oils were more likely to polymerize in the heat of the engine if used directly without trans-esterification. The peroxide value and p-anisidine value of all oils were in the recommended range. The flash point of all marine oils

Type of Fatty Acid	Crude Farmed Salmon Oil (Atlantic)	Crude Seal Oil	Crude Cod liver Oil	Crude Wild Salmon Oil (Pacific)
Saturated Fatty Acid				
Myristic acid (14:0)	3.20	4.23	3.11	4.52
Palmitic Acid (16:0)	12.79	7.69	9.60	13.88
Stearic Acid (18:0)	3.29	1.02	2.23	2.54
Other saturated fatty acids	0.55	0.59	1.10	1.59
Subtotal	**19.83**	**13.53**	**16.05**	**22.52**
Monounsaturated Fatty Acid				
Palmitoleic Acid (16:1n-7)	7.37	16.11	8.17	5.32
Oleic Acid (18:1n-9)	31.19	21.20	17.48	14.06
Vaccenic acid (18:1n-7)	3.44	4.76	5.36	3.15
Other monounsaturated fatty acids	5.38	16.81	22.78	22.44
Subtotal	**47.38**	**58.88**	**53.79**	**44.97**
Polyunsaturated Fatty Acid				
Linoleic acid (18:2n-6)	14.50	1.54	1.91	1.63
Alpha-linolenic acid (18:3n-3)	1.69	0.61	0.78	0.97
Arachidonic (20:4n-6)	0.61	0.49	0.38	0.63
EPA (20:5n-3)	4.63	7.12	8.52	9.54
DPA (22:5n-3)	0.13	0.09	0.11	0.12
DHA (22:6n-3)	3.48	8.45	11.36	10.55
Other polyunsaturated fatty acids	7.20	8.33	5.99	7.97
Subtotal	**32.23**	**26.64**	**29.05**	**31.42**

Table 4: Fatty acid composition (wt%).

were well above 200°C and therefore poses no risk of fire outbreaks in case of accidents. Slightly lower in cetane number was found after calculation in all marine oils except farmed salmon oil. However, they would increase after oil to biodiesel conversion.

All the marine oils have significant potential for use as feedstock due to higher (81%) triacylglycerol. A degumming process prior to biodiesel production will be required as the phospholipids (1.21-1.67%) were higher than the recommended limit of ≤10 ppm. All four marine oils have saturated (13.53-22.52%), monounsaturated (44.97-58.88%) and polyunsaturated (26.64-32.23%) fatty acids. All the studied marine oils have higher polyunsaturated fatty acids so biodiesel produced will have less oxidative stability and may result in the precipitation of biodiesel components in a fuel feeding system or combustion chamber. Therefore, it is crucial to stabilize oils and biodiesel immediately after extraction/production to obtain a high quality biofuel.

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