THE POTENTIAL OF FISH PROCESSING WASTES FOR BIODIESEL PRODUCTION

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EXECUTIVE SUMMARY

The following report represents the efforts of the Centre for Aquaculture and Seafood Development (CASD) research team in characterizing marine oils to identify its potential use as biodiesel feedstock and establish technology in the area of energy and waste management, enhance the competitiveness of Newfoundland and Labrador's biofuels and help Canada to meet its commitment to GHG emission building the renewable fuels.

To date, biodiesel is not readily available in Newfoundland and Labrador, and there are no biodiesel producers operating within the province. The scope of this project is the development of an *economically viable and environmentally sustainable biodiesel production system for rural communities in Newfoundland and Labrador* and to help marine processing plants cut down their operating cost, by diminishing the problem of fish waste disposal, and by providing alternative fuel for the operation of feed barges, marine vessels and generators located at their remote locations.

Crude cod *(Gadus morhua)* liver, Pacific salmon (*Oncorhynchus*) and Atlantic salmon (*Salmo salar*) oil were characterized to identify their suitability for biodiesel production. Since the feedstock oils used for biodiesel production are of diverse origin and quality, initial evaluation of the physical and chemical composition of the feed stock oil is very essential prior to biodiesel production. Investigation of physical properties (smell, color, physical state, moisture and specific gravity), chemical properties (pH, ash content, acid value, iodine value, saponification value, p-anisidine value, peroxide value, TOTOX value, free fatty acid, flash point, kinematic viscosity and refractive index) and lipid and fatty acids classification were performed on all marine oils.

The characterized marine oils were pale yellow to orange in color and were stable at 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 (0.057-0.771 mgKOH/g), peroxide (5.13-9.17 meq O_2/kg oil) and p-anisidine (3.36-9.67) values of all oils were within recommended limits, higher acid value in farmed salmon (2.441 mgKOH/g) and higher iodine value (116-139.15 g I_2/100 g). A drying step had to be implemented to remove the water because it can lead to corrosion of internal combustion engine components. Due to higher iodine value, 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 three marine oils were more likely to polymerize in the heat of the engine if used directly without transesterification. Flash point of all marine oils was above 200°C and there is no risk of fire outbreaks in case of accidents. Due to higher triacylglycerol (81-93%) content all the oils characterized in this study can be as a feedstock for biodiesel production via transesterification. Cod liver oil (14.72%) was 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 and require a degumming process 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.

1. INRODUCTION

1.1. Project Background and Rational

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 of marine landings and 163, 036 tons of aquaculture in 2012, 34% and 14%, respectively came from Newfoundland and Labrador (DFA, 2014). 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) (DFA, 2014a). In 2012, ***Salmonid production*** accounted for ***16,831 tons*** (79%) and was ***valued at $99 million***. Of the 145 licensed aquaculture plants, 84 produced. In 2012 cod production accounted for 8334 tons and was valued at $9.4 million (DFO, 2014b). 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 (Murugesan et al., 2009). ***Thus, it can be advantageous to develop byproduct applications that demand large volumes of fish waste, thereby making these industries viable and more environmentally friendly.***

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. If the utilization of fossil fuels is continued at the present rate, local air quality will deteriorate severely and ***global warming will increase beyond a repairable extent*** (Fukuda et al., 2001; Akoh et al., 2007). 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 (Demirbas, 2007). 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. In comparison to petroleum diesel, biodiesel significantly reduces emissions of carbon dioxide (about 50-60%), sulfur dioxide and harmful air pollutants, in particular asthma-causing soots. GHG emissions can be reduced by 10-20% and 40-90% with the use of at least 20% (B20) and 100% (B100) biodiesel blends, respectively (Hinton et al., 1999; Srivastava and Prasad, 2000; Subramanian et al., 2005).

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. 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*** (McNeff et al., 2008; Jegannathan et al., 2008; Ranganathan et al., 2008). ***These factors have necessitated the need for the development of a bio-***

refinery approach for production of biodiesel from waste based and cheap biomass rich in oil such as fish oils. This approach will result in sustainable biofuels and fishery industries.

To date, biodiesel is not readily available in Newfoundland and Labrador, and there are no biodiesel producers operating within the province. Newfoundland and Labrador's seafood industry generates on average ***102,850 tons (25% of Canada's fish waste)*** of processing discards from which valuable oils can be recovered. Newfoundland and Labrador has the potential to produce ***2600 tons of marine oils extracted from fish processing waste*** as the largest potential source of biodiesel feedstock that can be converted into approximately ***2,548 tons of biodiesel*** (assuming a 98% yield) (Manuel et al., 2006). Specific to salmonid industry waste, **360 tons** of fish oil can be extracted and utilized to produce biodiesel. Converting marine oil into biodiesel would benefit the marine industry sectors in reducing the disposal cost of theses waste to landfills and utilize biodiesel for operating feed barges, marine vessels and generators located at their remote locations. After the fish oil has been extracted, residual biomass can be used as a feed stock for production of biomethane and bioethanol via fermentation or fertilizer or feed for animals.

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 the oil extraction process (Bimbo, 2011). 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, moisture content, ash content, acid value, iodine value, saponification value, p-Anisidine value, peroxide value, free fatty acid, flash point, kinematic viscosity, refractive index (St. Angelo, 1996; Wrolstad et al.,2005; Boran et al., 2006; O'Brien, 2009).

1.2. Objectives

This project was built on past projects to advance current biodiesel conversion technologies for specific applications to marine waste oil feedstock in rural communities. Existing conversion technologies have been developed to handle primarily homogenous oils from vegetable sources. These technologies have had limited success when applied to marine waste oils. Mitigating the technical challenges specifically associated with utilizing marine waste oils as biodiesel feedstock will be the focus of the proposed project. The long term objective of the proposed research is to develop an economically viable, small to medium scale marine oil derived biofuel/biodiesel production system for aquaculture communities located in rural areas of Newfoundland and Labrador. This will enable aquaculture growers and processors to utilize their waste streams to produce biodiesel and operate feed barges, marine vessels and generators located at their remote locations.

In the present study, the physico-chemical characteristics of three crude marine oils including farmed salmon, cod liver and wild salmon are compared and interpreted with regard to their suitability as biodiesel feedstock. The short-term objectives were: (a) investigation of physical properties including smell, color, physical state, moisture and specific gravity (b) investigation of chemical properties

including pH, ash content, acid value, iodine value, saponification value, p-anisidine value, peroxide value, TOTOX value, free fatty acid, flash point, kinematic viscosity and refractive index (c) investigation of lipid classes and fatty acids.

The implemented project was funded through the Innovation OceanTech Intelligence Program, Department of Fisheries and Aquaculture and the Canadian Centre for Fisheries Innovation approved the proposal for year 1 (2011-2012) for the CASD project entitled "Demonstration Biorefinery for Waste Fish Oil". The research team had requested seed money from The Harris Centre – MMSB Waste Management Applied Research Fund to carry out components of year 2 (2013) research activities for pilot scale biodiesel production. Unfortunately funding support from other funders (IBRD, DFA, and CCFI) for year two research was not secured to carry out second year pilot scale research activities. Therefore, research activities were limited to only those components, (characterization of marine oils), for which funding **was received.**

1.3. Methodology

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 Ocean Sciences Centre of Memorial University of Newfoundland, Logy Bay, Newfoundland, Canada.

1.3.1. 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. Atlantic salmon (*Salmo salar*) oil was obtained from an oil extraction plant in Newfoundland, operated by Barry Group. Salmon oil was extracted from the waste stream from salmon guts obtained from local processing plants. Salmon oil was extracted using meat grinder, Contherm™ scraped-surface heat exchanger, 2-phase decanter centrifuge, 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 oils were stored in a dark place at room temperature (18-20°C) in tightly sealed containers.

1.3.2. Physical Properties

1.3.2.1. Smell, Color and Physical State

Odour, color and physical state of the oils were assessed by sensory evaluation.

1.3.2.2. Specific Gravity

Specific gravity was determined using a hydrometer (Model # 11582, Fisher Scientific, Ottawa, Ontario, Canada) at 16°C (60°F).

1.3.2.3. Moisture Content

The moisture 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 (Sandy Brae Laboratories, Inc., 2009).

1.3.3. Chemical Properties

1.3.3.1.1. pH

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

1.3.3.2. 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, 2008). Successive portions of oil were ashed after the initial ashing until the sufficient yield was obtained to calculate the percent ash.

1.3.3.3. 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. 50 mL of alcoholic potassium hydroxide was added to the oil sample using the pipette and it 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 reflex condenser on heating mantle. The boiled ethyl alcohol was distilled in a rotary evaporator and collected. 40 g of potassium hydroxide (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 was completely saponified. Complete saponification took place in approximately 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 jell 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 (ASTM D5558-95, 2011):

$$Saponification\ Value\ (mgKOH/g) = \frac{56.1 * N\ (A - B)}{wt\ of\ oil\ (g)} \quad (1)$$

Where:

N	=	Normality of HCl
A	=	Titration of blank (mL)
B	=	Titration of sample (mL)
56.1	=	Molecular weight of potassium hydroxide (KOH)

1.3.3.4. Free Fatty Acids and Acid Number

The free fatty acid content (%FFA) and acid number (AN) were determined using a colorimetric titration method similar to Rukunudin et al. (1998)**.** The test oil sample was filtered through a Whatman No. 40 filter paper to remove moisture and impurities. 1 mL of filtered oil sample was accurately weighed to 0.001 g and dissolved in 10 mL iso-propyl alcohol. A blank solution was simultaneously prepared without addition of oil. Three drops of phenolphthalein indicator were added to the sample and blank and titrated against 0.025 N until the pink color disappeared completely. The free fatty acids content (%FFA) and acid number were calculated using Equations 2 and 3.

$$FFA\ (\%) = \frac{(v - b) \times N \times 28.2}{w} \quad (2)$$

Where:

v	=	Volume of 0.025N NaOH needed to titrate sample (mL)
b	=	Volume of 0.025N NaOH needed to titrate blank (mL)
N	=	Normality of NaOH solution
W	=	Weight of oil (g)

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

1.3.3.5. 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. 0.16 g 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 mixed with a magnetic stirrer. Two blank solutions were also prepared simultaneously without addition of fish oil. 25 mL of Wijs solution was added and 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 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 yellow of the solution had disappeared. 1-2 Ml of starch indicator was added to the samples and the titration was continued until blue color of the solution had disappeared. The iodine value of the oil sample was calculated using the Equation 4.

$$Iodine\ Value = \frac{(B - S) * M * 12.69}{weight\ of\ oil\ (g)} \quad (4)$$

Where:

B = Titration of blank (mL)
S = Titration of test solution (mL)
M = Molarity of $Na_2S_2O_3$

1.3.3.6. Peroxide Value (PV)

The peroxide value was determined by AOAC official method Cd 8-53. The test oil sample was filtered through a Whatman No. 40 filter paper to remove moisture and impurities. 5 g of filtered oil samples were accurately weighed to 0.05 g in a 250 mL Erlenmeyer flask and 30 mL of 3:2 acetic acid-chloroform was added and mixed 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 left to stand for 1 min. 30 mL of distilled water was added to the oil samples and mixed with a magnetic stirrer. The samples were titrated against 0.1 N sodium thiosulfate until the yellow iodine color disappeared. 2 mL of starch indicator 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 titration value lesser than 0.5 mL and therefore the peroxide value determination was carried out using 0.01 N sodium thiosulfate.

$$Peroxide\ Value\ (milliequivalents\ peroxide/1000\ g\ sample) = \frac{(\mathrm{S}-\mathrm{B})*\mathrm{N}*1000}{\mathrm{W}} \qquad (5)$$

Where:

S = Volume of titrate sample (mL)
B = Volume of titrate blank (mL)
N = Normality of sodium thiosulfate solution
W = Weight of oil (g)

1.3.3.7. p-anisidine Value

The *p*-anisidine value was determined by AOAC official method Cd 18-90. The test oil sample was filtered through a Whatman No. 40 filter paper to remove moisture and impurities. 0.5-4 g of oil sample was 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 spectrophotometer (Jenway 6400/6405, Jenway Incorporated, Stone, Staffordshire, UK) with the solvent as blank. 5 mL of oil sample was pipetted into one test tube and 1 mL of p-anisidine reagent was added. 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 blank. After 10 minutes, the absorbance (A_S) of the oil sample with the p-anisidine reagent was measured at 350 nm using spectrophotometer. The p-anisidine value was calculated by using following Equation 6.

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

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)

1.3.3.8. TOTOX Value

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

1.3.3.9. Flash Point

The flash point was measured using a Pensky-Marten closed cup tester (K162XX, Koehler Instruments, Bohemia, New York, USA) according to the Procedure A in ASTM D93-13 (ASTM, 2012). 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 flashpoint was corrected for barometric pressure.

1.3.3.10. Kinematic Viscosity

The kinematic viscosity was measured with a Brookfield viscometer (DV-I, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a S61 spindle at 50 rpm.

1.3.3.11. Refractive Index

The refractive index was measured with a pocket ATAGO refractometer (PAL-S, ATAGO Co. Ltd., Tokyo, Japan) per manufacturer's instructions.

1.3.4. Lipid Class Determination and Fatty Acid Analysis

1.3.4.1. Lipid Extraction

Lipid samples were extracted according to Parrish (1999). 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 0.5mL of chloroform extracted water were added to the test tube. Chloroform extracted water was prepared by adding 1L of

distilled water in a separating funnel and 30 ml of chloroform was added to the water. 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 (Hooper and Parrish, 2009). 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 was 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 as previously described for three times and all the organic layers were pooled together. The lipid extracted was then evaporated under a gentle stream of nitrogen, sealed with Teflon® tape and stored in the freezer at -20°C until use.

1.3.4.2. Lipid Class Composition

Lipid class composition was determined using a Latroscan Mark VI TLC-FID (Latron Laboratories Inc., Tokyo, Japan), silica coated chromarods and a three-step development method (Parrish, 1987). The cromarods 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 solvents 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 the 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).

1.3.4.3. Preparation of Fatty Acid Methyl Esters (FAME) with H_2SO_4 in MeOH

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/100mL 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.

1.3.4.4. 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 autosampler. 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^{o}C at a rate of 40^{o}C/min for 15 minutes and again ramped to a final temperature of 220^{o}C at a rate of 2^{o}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^{o}C with hydrogen as a carrier gas at a flow rate of 2 mL/min. The injector temperature was started at 150^{o}C and ramped to a final temperature of 250^{o}C at a rate of 120^{o}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.

2. RESULTS

2.1. Physical Properties

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

Table 1: Physical properties of crude marine oils

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

2.1.1. Smell, Color and Physical State

All three marine oils had an agreeably oily smell, while cod liver and wild salmon had a slightly fishy smell. Cod liver oils were pale yellow in color, while farmed Atlantic salmon and wild Pacific salmon had orange and light orange colour, respectively. All marine oils were in clear liquid state at room temperature except wild Pacific salmon oil which was a cloudy liquid with small solid particles in suspension.

2.1.2. Specific Gravity

Specific gravity is the ratio of the density of the substance to that of water (1g/cm^3) at 15.6°C. It can be used to determine the purity of oils based on desired characteristics. Specific gravity of farmed salmon, cod liver and wild salmon oil was 0.921, 0.924 and 0.922 g/cm^3, respectively (Ibeto et al., 2012; Kywe and Oo, 2009). The higher viscosity of oils tends to cause problems including incomplete combustion and particulate matter emissions when used directly in diesel engines (Fort and Blumberg, 1982; Ziejewski et al., 1993).

2.1.3. Moisture Content

The moisture content of farmed salmon, cod liver and wild salmon oil was 325, 179 and 312 ppm, respectively. Obtained water content values were within the ASTM biodiesel standards (<500 ppm). The water content in oil must be within ASTM standards to prevent excessive soap formation during

chemical transesterification reaction. The produced soap also increases the viscosity of the reaction mixture, sometimes causing gel formation and trapping the resulting ester and glycerin together thereby making the separation of glycerol from ester difficult (Wright, 1944).

2.2. Chemical Properties

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

Table 2: Chemical properties of crude marine oils

S. No	Properties	Crude Farmed Salmon Oil (Atlantic)	Crude Cod liver Oil	Crude Wild Salmon Oil (Pacific)	ASTM specification for Biodiesel
1	pH	6.8	6.8	6.7	-
2	Ash content(%)	0.0045	0.0037	0.0034	0.02
3	Acid value (mgKOH/g)	2.441	0.057	0.771	0.8
4	Iodine value	116.79	139.15	138.79	120 (EN 14214)
5	Saponification value (mgKOH/g)	185.85	179.55	176.19	-
6	p-Anisidine value	3.36	6.20	9.67	-
7	Peroxide value (meq/kg)	9.17	6.92	5.13	-
8	TOTOX Value	21.69	20.03	19.92	
9	Free fatty acid (%)	1.23	0.03	0.39	< 2.5%
10	Flash point (°C)	244	227.0	208.5	130°C min
11	Kinematic viscosity (mm^2/s)	66.22	63.10	70.50	-
12	Refractive index	1.47	1.48	1.48	-

2.2.1. pH

The pH values for all three fish oils were a neutral in range of 6.5-6.8. The pH of feedstock oil is not a major factor in the base/acid catalyzed transesterification reaction. However, pH values are very important in the enzyme catalyzed transesterification reaction, because enzymes activity may get severely affected at lower or higher pH values.

2.2.2. Ash Content

Ash consists of the residue left when the fuel is heated to a sufficiently high temperature that combustible material burns and leaves as CO_2 and H_2O**.** The ash content of farmed salmon, cod liver and wild salmon oil was 0.0045, 0.0037 and 0.0034%, respectively. Obtained ash content of all three 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 amount of ash content in the feedstock oil tends to produce rejected quality of biodiesel according to ASTM standards (Van Gerpen et al., 2004).

2.2.3. Saponification Value

When oil and fat reacts with alkali their long chain fatty acid salts results into soap formation, glycerols and fatty acids. Soaps, which are the salts of longer chain fatty acids, are produced by treating a fat with alkali (Nielsen, 1998; Ockerman and Hansen, 2000). 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 a fat sample (Nielsen, 1998). In the current study, the saponification value of farmed salmon, cod liver and wild salmon oil was 185.85, 179.55, 176.19 mgKOH/g, respectively. Higher saponification values have indicated that both farmed and marine oils studied were comprised mainly of short chain fatty acids.

2.2.4. Free Fatty Acids

It is important to determine free fatty acids (FFA) content as it is used for quality criteria of fats and oils. Also, FFA is employed to assess fish deterioration during frozen storage and FFA content increases with increase in storage time. Higher amount of FFA limits their use for biodiesel production by acid-catalyzed and alkali-catalyzed methods. In case of biodiesel production by alkali-catalyzed method, maximum allowable is below 2.5 wt% FFA. Pretreatment step becomes very much essential if the oil or fat feedstock has a FFA content over 2.5 wt% (Leung et al., 2010). In this study, the free fatty acids (FFA) content in all three oils were relatively low compared to allowable limits and indicated that esterification prior to transesterification may not be necessary and a one-step reaction can complete the transesterification process and result in higher yield of biodiesel.

2.2.5. Acid Value

Oil acidity is an important quality parameter determining the presence of free fatty acid (FFA) and other non-lipid acid compounds (Rubio-Rodríguez et al., 2008). FFA is mostly generated by a hydrolysis reaction of triacylglycerides. As oils go rancid, triacylglyceride (TAG) coverts to fatty acid (FA) and glycerol increases the acid number. Acid value in feedstock oil should be less than 2.5mg KOH/g oil for the base-catalyzed transesterification process. However, acid value of oil should be less than 1 mg KOH/g oil to meet the alkaline catalyzed transesterification conditions. Recommended ASTM standard for acid value of biodiesel is 0.8 mgKOH/g (Leung et al., 2010). The acid values of cod liver and wild salmon oils were within the ASTM biodiesel standards (0.8 mgKOH/g) except for farmed salmon oil.

2.2.6. Iodine Value

The iodine number gives an indication about the amount of unsaturated fatty compounds (number of double bonds) in the oil and thereby an indication of 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 (Van Gerpen et al., 2004). As the number of double bonds does not change during transesterification, measuring the iodine value in feedstock oil already gives an indication of the stability of the biodiesel produced from same feedstock. Europe's EN14214 specification allows a maximum of 120 for the iodine number (g I_2/100 g) in biodiesel (Son et al., 2010). In this present study, the iodine value of farmed salmon, cod liver and wild salmon oil was 116.79, 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 are classified as drying oils; those with an iodine value of 110–140 are classified as semidrying oils; and those with an iodine value less than 110 are considered as non-drying oils. Cod liver and wild salmon oils were drying oils, while farmed salmon oil was semidrying oil.

2.2.7. Peroxide Value (PV)

The peroxide value is the measurement of primary oxidation product hydroperoxide and widely used chemical test for the determination of fats and oil quality (Aidos et al., 2001). 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 peroxide values of farmed salmon, cod liver and wild oil were 9.17, 6.92 and 5.13 meq O_2/kg oil, respectively. Acceptable PV values for fish oil were between 3 and 20. In this study, PVs of both examined oil samples did not exceed 20 meq O_2/kg oil.

2.2.8. p-Anisidine Value (AV)

The p-anisidine value is used to measure the secondary product 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 (IUPAC, 1987; O'Brien, 2009). The color obtained not only depends on the aldehyde 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 (St. Angelo, 1996). The p-*anisidine value* of farmed salmon, cod liver and wild salmon oil was 3.36, 6.20 and 9.67, respectively. All studied marine oils p-anisidine values are within the recommended range of ≤20 for crude fish oil (FAO and WHO, 2013).

The formation of primary and secondary degradation products will 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 alkaline catalyzed transesterification process for biodiesel production.

2.2.9. Totox Value

The 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 indicate an oil's overall oxidation state. Peroxide value can decrease over time so AV and/or Totox calculation are essential to get an understanding on total oxidation. FAO (2013) recommended Totox value is ≤26 for fish oil. In the present study all examined marine oils Totox values were within the recommended range.

2.2.10. 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 (Gmehling and Rasmussen, 1982). Flash point of fall examined marine oils was well above 200°C. They were all above the 130°C minimum with respect to the minimal flash point regulated for biodiesel according to ASTM norm D6751 and therefore pose no risk of fire outbreaks in case of accidents.

2.2.11. Kinematic Viscosity [cP]

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 a combustion process. Previously published data revealed a direct relationship between the oil viscosity and some chemical characteristics of the lipids including the degree of unsaturation and the chain length of the fatty acids that constitute the triacylglycerols. Viscosity slightly decreases with increased degree of unsaturation and rapidly increases with polymerization (Abromovic andKlofutar, 1998; Stanciu, 2011). In the present study, the kinematic viscosity of all marine oils is above the range allowed by ASTM standard D975 between, 1.9- and 6.0 cSt., respectively.

2.2.12. Refractive Index

Refractive index is used to measure the increase on 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 index and peroxide values of the oils and fats can significantly increase while exposed to light and heat (Arya et al., 1969). In this study, the refractive index of farmed salmon, cod liver and wild salmon oils were 1.47, 1.48, 1.48 and 1.48, respectively.

2.3. Lipid Classification

Lipid classes in the farmed salmon, cod liver and wild salmon oil are presented in Table 3. Among the lipid classes, triacylglycerol, free fatty acid, phospholipids, sterols and polar lipids were predominant constituents, while hydrocarbons were minor components in all the marine oils studied. Hydrocarbons, sterol esters and triacylglycerol were the major components, of neutral lipids from different marine oils. The presence of higher percentage (more than 80%) of triacylglycerols were found in all marine oils including farmed salmon (92.83%), cod liver (85.26%) and wild salmon (81.20%), which is essential for transesterification of feedstock oil and higher biodiesel production yield. Hydrocarbons content was absent in the farmed and wild salmon oil, while 0.33% was present in the cod liver oil. Farmed salmon, cod liver and wild salmon oil was also comprised of 3.64, 3.24 and 2.02% of sterol esters, respectively. Cod liver (14.72%) was rich in polar lipids while the farmed salmon (2.43%) and wild salmon (2.43%) were low in polar lipids. Phospholipids (Gums) in oils refer to hydratable and non-hydratable phosphatides, lecithin, sugars, trace metals and other impurities (McDonnell et al., 1995; Indira et al., 2000). For biodiesel producers, gums are a concern for following reasons: (a) inhibit the catalyst during the transesterification reaction (b) difficult to separate biodiesel and glycerol due to the emulsifying effect that occurs after the transesterification reaction (c) metals contamination in the final product (P and Ca specifically), (d) yield loss and (e) ASTM D 6751-09 requires the maximum amount of 10 ppm phosphorus content in the final biodiesel fuel (Freedman et al., 1984; Fan et al., 2010). In the present study, higher amounts of phospholipids were observed in farmed salmon oil (1.43%) which requires a degumming process prior to biodiesel production.

Table 3: Composition of marine oils lipid classes

S. No	Parameter	Crude Farmed Salmon Oil (Atlantic) (%)	Crude Cod liver Oil (%)	Crude Wild Salmon Oil (Pacific) (%)
1	Hydrocarbons	0.00	0.33	0.00
2	Steryl Esters/Wax Esters	0.00	0.00	0.00
3	Ethyl Esters	0.00	0.00	0.00
4	Methyl Esters	0.00	0.00	0.00
5	Ethyl Ketones	0.00	0.00	0.00
6	Methyl Ketones	0.00	0.00	0.00
7	Glycerol Ethers	0.00	0.00	0.00
8	Triacylglycerols	92.83	85.26	81.20
9	Free Fatty Acids	1.33	0.05	0.40
10	Alcohols	0.00	0.00	0.00
11	Sterols	3.64	3.24	2.02
12	Diacylglycerols	0.00	0.00	0.00
13	Acetone Mobile Polar Lipids	2.43	14.72	2.43
14	Phospholipids	1.43	1.21	1.67

2.3.1. 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, cod liver and wild salmon oil consisted of 19.83, 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, cod liver and wild salmon oil had also comprised of 47.38, 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. 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 farmed salmon, cod liver and wild salmon oil comprising of 32.23, 29.05 and 31.42%, respectively. Biodiesel produced from saturated fat had the higher cetane number and improved oxidative stability; however, higher cloud point and poor temperature properties like formation of gel at ambient temperature. If more polyunsaturated fatty acids are present in the feedstock oil then reduced cloud point, cetane number and stability can be observed in the biodiesel. In the present study, all marine oils have higher polyunsaturated fatty acids, so biodiesel produced will have less oxidation stability and may result in the precipitation of the biodiesel components in a fuel feeding system or combustion chamber (Tanwar et al., 2013; Pinyaphong et al., 2011; Ramadhas et al., 2005).

Table 4: % Fatty acid composition

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

3. SUMMARY AND CONCLUSION

Mitigating the technical challenges specifically associated with utilizing marine waste oils as biodiesel feedstock was the focus of the proposed project. The main aim of the project was to address challenges related to the utilization of waste fish oil prior to pilot scale biodiesel production. All marine oils were extracted from the waste stream(s) obtained from fish processing plants. All oils were extracted using a meat grinder, contherm™ scraped-surface heat exchanger, 2-phase decanter centrifuge, Westfalia polishing centrifuge (*this oil extraction equipment is available at the Marine Bioprocessing Facility at the Marine Institute, however installation of the contherm™ scraped-surface heat exchanger, 2-phase decanter centrifuge is incomplete due to insufficient funding*). Based on this study, all characterized oils have potential use as biodiesel feedstock with proper handling and storage, and implementation of pretreatment for removal of free fatty acids and phospholipids. The study also shows that the oxidative stability of feedstock depends on the presence of natural antioxidants, degree of unsaturation, and branching of the chain. Moreover, the stability of biodiesel produced can also depend on the biodiesel production process used.

The physico-chemical characteristics of three marine oils from farmed Atlantic salmon, cod liver and wild salmon were compared and interpreted with regard to their suitability as biodiesel feedstock. The lipid classification and the fatty acid composition of all three marine oils were also performed.

1. The physical properties of three crude marine oils including farmed Atlantic salmon, cod liver and wild salmon oil were determined and the following results were obtained:

    a) All oils were pale yellow to orange in color and were stable at liquid state at room temperature;
    b) Specific gravity of farmed salmon, cod liver and wild salmon were 0.921, 0.924 and 0.922 g/cm^3, respectively which were significantly closer to the biodiesel standard range of 0.87–0.90;
    c) The moisture content of farmed salmon, cod liver and wild salmon were 325, 179 and 312 ppm, respectively;
    d) The water content value of all the marine oils were within the ASTM biodiesel standards (<500 ppm).

2. The chemical properties of crude marine oils including farmed salmon, cod liver and wild salmon were determined and the following results were obtained:

    a) The pH values of all crude marine oils were neutral in range of 6.5-6.8;
    b) The ash content of all the three marine oils was in the range of 0.0027-0.0045%, respectively;
    c) Obtained ash content of all oils was much lower than the ASTM biodiesel standards (0.02%);
    d) The saponification value of all three marine oils was in the range of 176.19 and 185.85 mgKOH/g, respectively;
    e) Higher saponification values have indicated that all marine oils studied were comprised mainly of short chain fatty acids.

f) The free fatty acids (FFA) content of all three marine oils were in the range of 0.03-1.23%, respectively and relatively low compared to allowable limits of 2.5%;
g) The acid value of farmed salmon, cod liver and wild salmon was 2.441, 0.057 and 0.771 mgKOH/g, respectively;
h) The iodine value of farmed salmon, cod liver and wild salmon was 116.79, 139.15 and 138.79, respectively. The farmed salmon oil was semidrying oil and the other two oils were characterized as drying oil;
i) The peroxide value of farmed salmon, cod liver and wild salmon was 9.17, 6.92 and 5.13, respectively;
j) The p-anisidine value of all three marine oils was in the range of 3.36-9.67, respectively. All studied marine oils p-anisidine values are within the recommended range of ≤20 for crude fish oil;
k) Flash point of all three marine oils was well above 200°C. They were all above the minimum requirement of 130°C and therefore pose no risk of fire outbreaks in case of accidents;
l) The kinematic viscosity of all three marine oils was in the range of 63.10-70.50 mm2/s, respectively which is the higher than the allowed range of 1.9-6.0 cSt by ASTM standard D975;
m) The refractive index values of all three marine oils were in the range of 1.47-1.48, respectively.

3. The lipid classification of all oils was carried out. Among the lipid classes, triacylglycerol, free fatty acid, phospholipids, sterols and polar lipids were predominant constituents, while hydrocarbons were minor components in all the marine oils studied.

a) The farmed salmon, cod liver and wild salmon were comprised of 92.83, 85.26 and 81.20% triacylglycerols, respectively.
b) The presence of higher percentage (more than 80%) of triacylglycerols is essential for transesterification of feedstock oil and higher biodiesel production yield.
c) Hydrocarbons were absent in the farmed salmon oil and wild salmon whereas cod liver oil had 0.33%.
d) Farmed salmon, cod liver and wild salmon were also comprised of 3.64, 3.24 and 2.02% of sterol ester, respectively.
e) Higher amounts of phospholipids observed in farmed salmon oil (1.43%), cod liver (1.21%) and wild salmon (1.67%) require degumming process prior to biodiesel production.
f) 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%.
g) Farmed salmon, cod liver and wild salmon were comprised of 19.83, 16.05 and 22.52% of saturated fatty acids.
h) Farmed salmon, cod liver and wild salmon were comprised of 47.38, 53.79 and 44.97% of monounsaturated fatty acids.
i) Farmed salmon, cod liver and wild salmon were comprised of 32.23, 29.05 and 31.42% of polyunsaturated fatty acids.

j) In the present study, all three marine oils have higher polyunsaturated fatty acids so biodiesel produced will have less oxidation stability and may result in the precipitation of the biodiesel components in a fuel feeding system or combustion chamber.

4. FUTURE WORK AND RECOMMENDATION

The final report will be submitted to project partners. Benefits specific to a given partner will be highlighted and the implementation of results/procedures/know how will be discussed. Possible future collaboration will be explored and applications for new funding will be discussed.

Any further cooperation in this project or future projects will be discussed with individual partners. It is anticipated that this current cooperation will continue and strengthen. The present work has the potential to grow and show capabilities in the area of biotechnology and waste management. Bioprocessing/biorefinery strategies can be implemented which use fisheries and aquaculture waste to produce biodiesel, chitin/chitosan and other value-added products (biodiesel and high value nutraceuticals (omega-3 fatty acids), pharmaceuticals (chymotrypsin, pepsin, collagenase, enzyme inhibitors: alpha-1 antiproteinase, alpha-2 macroglobulin, anticoagulants: heparin and heparin-like glycosaminoglycans), high value proteins and meal; and bioconversion of glycerol into propanediol, ethanol and lactic acid.

5. KNOWLEDGE MOBILIZATION AND DELIVERABLES

As a part of knowledge mobilization, Dr. Deepika Dave presented some of the research findings at the following conferences.

- Dave, D. (2014). Bioprocessing strategy for the production of value added products from marine processing waste: A Biorefinery approach for sustainability. Global Change Biology- B4307, Marine Institute, St. John's, Newfoundland and Labrador, Seminar given on March 28, 2014.
- Dave, D. (2014). Bioprocessing strategy for the production of value added products from marine processing waste: A Biorefinery approach for sustainability. Cold Harvest 2014, 21st Annual Conference and Trade Show, Gander, Newfoundland and Labrador, February 11-13, 2014.
- Dave, D. (2013). Bioprocessing strategy for the production of biodiesel from Salmon byproducts: A Biorefinery approach for sustainability. Earth Bound Newfoundland and Labrador Organic Waste Conference, September 26-27, 2013.
- Dave, D. (2013). Bioprocessing strategy for the production of biodiesel from salmon byproducts: A Biorefinery approach for sustainability. Cold Harvest 2013, 20th Annual Conference and Trade Show, Gander, Newfoundland and Labrador, February 19-21, 2014.

5.1. Deliverables

- D. Dave, "The potential of fish processing wastes for biodiesel production", final project report to the Harris Centre at Memorial University (2014).
- D. Dave, S. Trenholm, V. V. Ramakrishnan, J. Pohling, H. Manuel and W. Murphy (2014). Comparative study of the physicochemical characterization of marine oils as potential feedstock for biodiesel production. Journal of Bioprocessing & Biotechniques. (Manuscript prepared and ready to submit).
- D. Dave, S. Trenholm, V. V. Ramakrishnan, H. Manuel and W. Murphy (2014). Optimization of degumming methods and evaluation of fuel properties. (Under preparation).

6. FIGURES: BUDGET ALLOCATIONS

Account: 80990
Total award: $15,000
70% of funds released =$10,500.00 upon approval
30% of funds released = $ 4,500.00 upon receipt of final deliverables

07/06/2013-05/09/2013	Research Assistant Salaries and Employee Benefits:	$13,413.66
02/07/2013-20/02/2013	Materials and Supplies:	$ 1,634.74
	Total:	$15,000.00
	Balance:	$ 4,500.00*

Note: *Available balance includes commitments.

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