BIOFUELS FROM FISH WASTE FROM REMOTE FISH PROCESSING PLANTS IN NEWFOUNDLAND AND LABRADOR

PUNYAMA JAYASINGHE

Biofuels from Fish Waste

From Remote Fish Processing Plants

In Newfoundland and Labrador

Punyama Jayasinghe, B. Sc. Eng (Hons)

A thesis submitted to the

School of Graduate Studies

in partial fulfilment of the

requirement for the degree of

Master of Engineering

Faculty of Engineering and Applied Science

Memorial University of Newfoundland

St. John's, Newfoundland, Canada

November, 2010

Abstract

Biofuels derived from waste and recycled oils are gaining attention throughout the world. Deriving biofuels from fish waste and use on-site can have a number of advantages in areas of substantial fish processing such as Newfoundland and Labrador. Currently, the waste is sent to landfill and/or discharged to the ocean. Depending on the fish species, between 3-25% of the waste is oil. However, composition, stability, degree of processing required, and end use will determine feasibility of use. Fish processing plants in Atlantic Canada are remotely located, making recovery of the oil for export for fuel use unattractive economically or environmentally. On-site use is likely the most sustainable option for reducing the impacts of waste discharge and, reducing emissions and costs for petroleum fuels use and transport.

The study is conducted to determine the feasibility and impacts of using fish waste derived biofuel as a blend for use on-site, in the community, or in marine vessels. Waste from three fish processing plants was characterized for chemical composition, stability, and partitioning. A process to separate and purify the oil from the waste was developed by modifying the fishmeal process. Recovered oil was analyzed for physical properties such as; density, viscosity, melting properties and specific heat capacity, and chemical composition was analyzed for sulphur content, lipid classes, and fatty acids. Using energy consumption and oil recoverability data for the proposed process, an overall life cycle analysis is conducted for estimating reductions in gaseous and GHG emissions, and solid/liquid waste discharge to the ocean. Emission studies were carried out for in-plant use in furnaces, stationary diesel engines and residential boilers.

Acknowledgement

The research was made possible with the support of a number of people. First of all, I would like to extend my sincere gratitude to the driving force behind the research and my supervisor, Dr. Kelly Hawboldt, for her untiring guidance and encouragement, and also the opportunity to work on a research of my passion. Under her direction I had the opportunity to conduct experiments at many laboratories with the support of academics from various disciplines. To begin with, I would like to express my appreciation to Dr. Christina Bottaro and Dr. Greet Van Beisen from the Department of Chemistry for providing resources and valuable inputs to the research. Special thanks go to Dr. Christopher Parrish, Jeanette Wells and Adrianus Both at the Ocean Sciences Centre for the extensive support and direction given for the lipid analysis. I also express my profound gratitude to Heather Emmanuel and Wade Murphy at the Centre for Aquaculture and Seafood Development, of the Marine Institute for arranging the use of facilities and obtainment of fish processing by-products. The efforts of Trevor Thomas from the, Department of Fisheries and Aquaculture is appreciated for acquiring fish processing by-products. The extension of resources/ for various sections of the research by; Nancy Leawood (CREAIT), Dr. Sue Ziegler (Earth Sciences), and Dr. Cynthia Coles (Engineering) are also greatly appreciated. I would also like to extend a special note of thanks to all the professors, post doctorial fellows and colleagues at the Department of Process Engineering for their advice, support and encouragement. I am also highly thankful to Dr. George Mann for introducing me to Dr. Hawboldt. I am forever indebted to my parents, my aunt and uncle for bringing me up. Finally, I would like to sincerely thank my husband Gayan Gamage for his encouragement in times of need and support in every possible way to carry out my research.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AKTS	Advanced Kinetics and Technology Solutions
ALC	Alcohols
AMPL	Acetone mobile polar lipids
ANOVA	Analysis of variance
AOAC	Association of Official Agricultural Chemists'
AOCS	American oil chemists' association standards
ASTM	American Society for Testing and Materials
B20	Biodiesel with 20% biodiesel in blend with petroluem diesel
B40	Biodiesel with 40% biodiesel in blend with petroluem diesel
B60	Biodiesel with 60% biodiesel in blend with petroluem diesel
B100	Biodiesel with 100% biodiesel in blend with petroluem diesel
$Ba(OH)_2$	Barium hydroxide
BF_3	Boron trifluoride
BOD_5	Biological Oxygen Demand
C	Carbon
Ca	Calcium
$Ca(OH)_2$	Calcium Hydroxide
Cd	Cadmium
$CaCl_2$	Calcium chloride
CFPP	Cold filter plugging point
$(CH_3)_3N$	Hydrogen sulfide and trimethylamine
CH_4	Methane
CO	Carbon Monoxide
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
DHA	Docosahexaenoic acid
DO	Dissolved oxygen
DSC	Differential scanning calorimetry
EF	Emission factor
EPA	Eicosapentaenoic acid
EU	European Union
FAME	Fatty acid methyl ester
FAO	Food and agricultural organization
$FeCl_3.\ 6H_2O$	Ferric chloride hexahydrate
FFA	Free fatty acids
FID	Flame ionization detector
FOG	Fats, Oil and Grease
FTIR	Fourier Transform Infrared Spectroscopy
GLC	Gas liquid chromatography
GC	Gas chromatography
GHG	Green house gas emissions
H	Hydrogen
H_2	Hydrogen gas
H_2SO_4	Sulphuric acid
$H_3(PO)_4$	Phosphoric acid

HAZMAT	Hazardous Materials
HC	Hydrocarbons
HCl	Hydrochloric acid
Hg	Mercury
HPLC	High performance liquid chromatography
IV	Iodine value
KET	Ketone
KH_2PO_4	Potassium dihydrogen phosphate
K_2HPO_4	Dipotassium hydrogen phosphate
KOH	Potassium hydroxide
LC_{50}	Lower concentration that destroys 50% of the test population
LCA	Life cycle analysis
Mg	Magnesium
MgO	Magnesium Hydroxide
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
m/m%	Percentage by mass
MS	Mass spectrometry
MUFA	Monounsaturated fatty acids
N	Nitrogen
N (before solutions)	Normality or gram equivalents of solute in the solution
NaOH	Sodium Hydroxide
$Na_2HPO_4.7H_2O$	Disodium hydrogen phosphate heptahydrate
NB	New Brunswick
NH_4Cl	Ammonium chloride
NIR	Near infrared spectroscopy
NL	Newfoundland and Labrador
NMR	Nuclear magnetic resonance
N_2O	Nitrous oxide
NO	Nitrogen oxide
NOx	Nitrogen dioxides
NS	Nova Scotia
P	Phosphorous
PAH	Poly aromatic hydrocarbons
Pb	Lead
PCB	Polychlorinated biphenyl
PL	Phospholipids
PTFE	Polytetrafluoroethylene
Ppm	Parts per million
PM	Particulate matter
PUFA	Polyunsaturated fatty acids
QCA	Quench cooling accessory
SDE	Stationary diesel engine
SE	Steryl esters
RMA	Marine Residual fuel type A
RMB	Marine Residual fuel type B
R-sq	Regression R-squared values
RSD	Relative standard deviation
S	Sulphur

SFA	Saturated fatty acids
SO_2	Sulphur Dioxide
SOx	Sulphur Dioxides
ST	Sterols
TAG	Triacylglycerols
TBHQ	Tertiary butylhydroquinone
TDS	Total dissolved solids
TGA	Thermo gravimetric analyzer
TLC	Thin Layer Chromatography
TSS	Total Suspended solids
TS	Total solids
UK	United Kingdom
USA	United States of America
VS	Volatile solids
v/v%	Percentage by volume
WE	Wax esters
wt. %	Percentage by weight
Zn	Zinc

List of Symbols

f	Flow behaviour index of recovered product or oil
σ	Shear stress of recovered product or oil
γ	Shear rate of recovered product or oil
π	Pi or the equivalent value of 22/7
ρ_E	Bulk density of effluent/waste samples
ν	Kinematic viscosity of recovered product or oil
μ	Dynamic viscosity of recovered product or oil
P_L	Percentage lipid or lipid class content in the effluents/waste
P_{LO}	Percentage total/classes lipid content in recovered product or oil
P_{YR}	Percentage recovered product or oil
V_E	Volume of effluent/waste samples
W_E	Weight of effluent/waste samples
W_o	Weight of recovered product or oil

Chapter 1

Introduction

1.1 Background

Biofuels may be derived from food crops or waste oil. Waste oils from processing of greases and animal fats can be used to derive biofuels for direct use in applications where the quality of the fuel required is lower and/or the engine is flexible to accommodate low quality oils. Waste oil derived biofuels have a number of advantages over conventional petroleum based fuels; the waste oil is used as a by-product instead of being disposed and the use of waste oil derived biofuels overall lowers GHG emissions and most other toxins (excluding nitrogen oxides) over the life cycle of fuel production, use and disposal.

The fish processing industry is an important part of rural and remote communities in Atlantic Canada, and generates waste in the amounts of 418,000 t/y [1]. Valuable by-products from fish processing waste can include; fish oil (ω-3 fatty acids), chitosan, chitin, cosmetics, natural pigments, animal feed, and soil fertilizers [2]. Generation of fish waste in Newfoundland and Labrador (NL) in the amount of 35,000 t/y. Excluding seal processing plants and fish meal plants (2 sites), ocean disposal of the waste is permitted due to the remote locations [1]. Capelin, herring, mackerel, seal and farmed salmanoids are the most common species processed [3]. The effluents consist of viscera, bones, liver, belly trimmings, kidney and skin, which are mixed with wastewater before discharging. The plants are remote as outlined in Figure 1.1 and transportation of by-products needs to be minimized for useful/effective recovery.

Figure 1.1: Location map of fish processing plants in NL (adopted from [1])

Table 1.1 indicates that a high amount of total suspended solids (TSS) and fats, oil and grease (FOG) can be present in the effluent [1, 4-7]. Discharge of the waste to the marine environment may result in anoxic conditions and decreased light penetration. The build up of organic material in the sediment through particle settling reduces the diversity of the fauna and flora in the long term. Recovery of part or all of the oil present in the waste can reduce the impact to the marine environment. The fat content of the virgin fish discards is estimated to be in the rage of 3.8% to about 25% [3]. With a 75% recovery, this translates to about 965 t/y of oil in the waste. High (1 – 5 wt.%) free fatty acid (FFA) content, heterogeneity of the waste and remoteness make processing to recover edible oils, transport to a central facility or export less feasible as by-product processing options.

Table 1.1: Properties of fish processing plant waste water (adopted from [1, 4 - 7])

Species/ plant type	TSS (mg/L)	COD (mg/L)	BOD (mg/L)	FOG (mg/L)
Herring	600-5,000	1,200-6,000	150	600-800
Tuna	180-1,000	700-1,640	125-900	90-600
Salmon	120-5,500	300-5,500	250-2,500	44-1,700
Marine finfish	100-900	100-800	600-2,000	80-2,480
Ground fish	300-1,500	100-2,000	200-1,200	200-1,500

TSS – Total suspended solids, COD – Chemical oxygen demand, BOD – Biological oxygen demand, FOG – Fats, oils and Grease

Processing on site and use in applications requiring low quality oils would likely create energy savings and also environmental and economic benefits. Use as a fuel for the processing plant's boiler or furnace, home heating oil in the community or even fish processing vessels are some of the possible applications. The present research is focused on studying the recoverability of the oil and the degree of processing required for producing biofuels from fish processing plant effluents in NL. Additionally, a life cycle analysis is conducted for solids and gaseous emissions.

1.2 Research objectives and scope

The fish processing industry in NL discharges a substantial amount of waste to the ocean/landfills. The high oil content and current challenges in recovering high end by-products make the option of recovering the oil for on-site use as a fuel blend attractive. In addition to waste reduction, substituting part of the petroleum based fuel with biofuel could reduce costs and emissions. The purpose of this work is to determine the feasibility and impacts of using fish waste derived biofuel pure and/or blended for use on-site or in the community to aid in the sustainability of rural communities in NL.

The specific objectives include:

1. Characterization of the fish waste in terms of; chemical composition, stability, reactivity and partitioning, by obtaining effluent samples from different fish processing plants as a pilot study.

   Fish processing waste oil can have low thermal and oxidation stability [8, 9].

2. Determine the recoverable oil and, the chemical composition, and stability of the recovered oil.

   The fats, oil and grease content are high as outlined in Table 1.1, but, this varies between the types of fish processed. While not many studies have defined the lipid composition, it has been shown that triacylglycerides (TAG) are the predominant class present [10, 11, 12]. The polyunsaturated fatty acids (PUFA) in marine species would mean the recovered oil would have low stability [3]. Degradation of the oil can also result in high FFA content.

3. Determine the minimal degree of processing required followed by the optimal blends and requirements for use as a biofuel for identified on-site applications.

   The degree of treatment required would depend on the end use.

4. Perform life cycle analysis of the process for reductions in gaseous/GHG emissions and solid/liquid waste.

   Recovering the oil and use on site can have reductions in emissions, and solid/liquid wastewater discharge [13, 14].

1.4 Contribution of the thesis

Recovering the oil can reduce the environmental impacts to the marine environment. Using part or all of the oil onsite can reduce costs associated with diesel use and reduce CO_2, CO, SO_2 and Particulate matter (PM) emissions. Overall the research contributes to the sustainability of the rural communities of NL.

1.5 Thesis organization

The thesis begins with key findings from the literature review, where Chapter 2 provides a general overview into biofuels by discussing the potential biomass feedstock, chemical/ thermal/ physical properties of biofuels derived from virgin and waste sources, conversion processes (recovery, purification and refining) used for biofuels from animal fats (beef tallow, lard, and poultry waste) and greases, and resulting performance as a fuel. Chapter 3 addresses aspects specific to biofuels from fish waste, such as; composition of the fish waste, oil content, recovery/refining processes and properties of recovered oil. In addition, case studies for crude fish oil/fish biofuel in engines are outlined, including engine performance and emissions, in comparison to petroleum fuels. Chapter 4 describes the methodology used for; characterization of the waste (chemical composition), recovery and purification of oil, and characterization of the oil (chemical composition and physical/thermal properties). Results and Analysis are given in Chapter 5, and Chapter 6 provides a discussion of the experimental results. Chapter 7 covers the life cycle analysis of the proposed process in terms of GHG and gaseous emissions and solid/liquid waste reductions. Chapter 8 summarizes the overall conclusions and recommendations from the study.

Chapter 2

Literature Review – Biofuels from Biomass

2.1 Introduction to bio-oils

Plant matter, animal waste, agricultural crops and residues, municipal waste, and industrial effluents are all possible biomass feedstock for deriving biofuels [15, 16]. Bio-gas, bio-oil and bio-char are the three main forms of biofuels and are typically converted from biomass via biochemical, thermochemical, physical, and chemical processes [17, 18]. Figure 2.1 presents, different biofuel forms produced and potential end uses. Bio-gas is produced via gasification, pyrolysis and anaerobic digestion [17, 18, 19]. Partial gasification with steam or CO_2 or a mixture of both, pyrolysis and, chemical activation processes are used for bio-char production [19]. Bio-oils are produced using pyrolysis, fermentation, hydrolysis and physical or chemical extraction processes [18, 20].

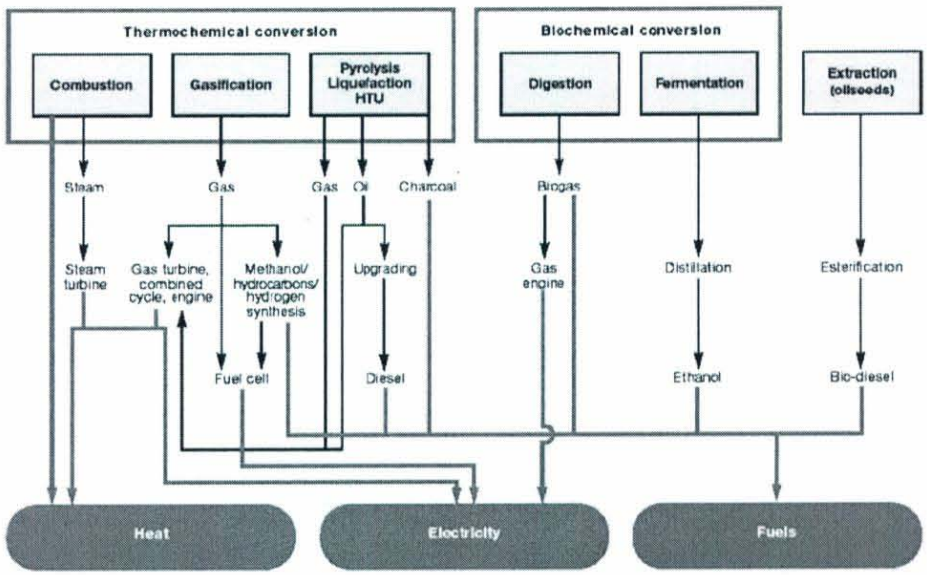


Figure 2.1: Biomass conversion processes and products [17, 18]

Bio-oils are derived from biomass such as; edible and non edible oilseed crops, wood (lignocelluloses), microorganisms, algae, animal waste and recycled cooking greases [16, 21]. Rapeseed, soybean, palm, sunflower, corn, safflower, canola, mustard, jatropha and mahua are the most common virgin crops for bio-oil production [21, 22, 23, 24]. Wheat, maize, sugar beet and potatoes are used for bioethanol production [15]. However, the food vs. fuel debate and other environmental impacts related to cultivation and conversion of the crops to fuels, present challenges to the use of food crops for fuel. Utilization of unproductive land and low quality edible oils produced for human food consumption and growth as a rotation crop address some of the issues [21]. The conversion of residues from wood or lignocelluosic material to biofuels is difficult, and advanced technologies are expected to reach their commercial stage in the next decades [25]. Yeast, bacteria, fungi and mould do not require land for production and, the lipid content of yeast and mould can be up to 70% [16]. Microalgae are another option as the oil per unit area is thirty times higher for algae than terrestrial oil seed crops [21]. However, large scale oil production issues are associated with both microalgae and microorganisms [16]. Biofuels from waste biomass has the advantage of recovering a valuable by-product from a waste stream thereby decreasing the volume of waste and toxicity.

Waste biomass associated with the food industry includes yellow grease, brown grease, beef tallow, meat and poultry waste, and fish waste [21]. Yellow grease is waste oil from restaurants using deep frying oils and is used as an animal feed, limiting its use as a fuel. Brown grease from restaurant grease traps is high in contaminants and degraded products; requiring treatment through several mechanical, thermal and chemical treatment processes and limiting potential energy benefits in its use as a fuel. By-products from poultry and fish

processing industries are also utilized for producing fats and oils in addition to protein meals. Beef tallow is a by-product of cattle slaughter plants [26]. Bovine spongiform encephalopathy has limited the use of beef tallow for other uses, therefore, both chemical conversion to bio-oils and direct firing, are options. One of the key parameters in use as a fuel is the type of biofuel produced. In the proceeding sections the focus is on bio-oils.

2.1.1 Composition of bio-oils

Fats and oils derived from biomass are known as lipids, and include fatty acids, their derivatives, and substances related to their compounds [27]. Although classes of lipids are common to all species; the fatty acid composition is a function of the origin (plant, animal, aquatic species or microbial) [27]. Triacylglycerols and related compounds (diacylglycerols, monoacylglycerols), hydrocarbons (HC), ketones (KET), alcohols (ALC), cholesterol, sterols (ST), wax esters (WE), free fatty acids (FFA), glycero-phospholipids, glycerol-glycolipids, etherlipids and sphingolipids are some lipid classes in animal and plant tissues [27, 28].

The TAG are the key lipid fraction of interest in the use of bio-oil as a fuel. A TAG is comprised of three fatty acids with a glycerol molecule (Figure 2.4). TAG and other lipid molecules decompose and form free fatty acids (FFA) [16]. High FFA levels (>5%) limit the waste fat/oil as a fuel due to high acidity. Recycled oils and animal fats have up to 20% FFA content, due to decomposition from steam, salts, chemicals and heat in processing or cooking [16, 21]. Refined canola and soybean oil have FFA less than 1.5% while greases and animal fats can range from 1.5% to over 20% specifically yellow grease has less than 15% FFA while brown grease levels are typically greater than 15% [21, 29]. Some vegetable oils also have high FFA, for instance mahua oil has a FFA of 20% [22].

Fatty acid content can be between 94 and 95 wt. % of a TAG molecule [29]. The type of fatty acid can vary by the carbon chain length, level of saturation of the carbon bonds and the number of unsaturated carbon bonds [16]. The degree of saturation determines the category of fatty acid; saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids. Table 2.1 outlines the structure and names of the fatty acid where the number of carbons and degree of saturation are indicated (e.g. C12:1 has 12 carbon atoms with one unsaturated bond) and the ω sign indicates the position of the double bond.

Table 2.1: Systematic and trivial names and structures of fatty acids [16, 24, 11, 30, 31]

Structure	Trivial name	Systematic name	Formula
C12:0	Lauric	Dodecanoic	$C_{12}H_{24}O_2$
C14:0	Myristic	Tetradecanoic	$C_{14}H_{28}O_2$
C14:1	Myristoleic	Tetradecenoic	$C_{14}H_{26}O_2$
C15:0		Pentadecanoic	$C_{15}H_{30}O_2$
C15:1		Pentadecenoic	$C_{15}H_{28}O_2$
C16:0	Palmitic	Hexadecanoic	$C_{16}H_{32}O_2$
C16:1	Palmitoleic	9-Hexadecanoic	$C_{16}H_{30}O_2$
C17:0	Margaric	Heptadecanoic	$C_{17}H_{34}O_2$
C17:1		Heptadecenoic	$C_{17}H_{32}O_2$
C18:0	Stearic	Octadecanoic	$C_{18}H_{36}O_2$
C18:1ω9	Oleic	9-Octadecenoic	$C_{18}H_{34}O_2$
C18:2ω6	Linoleic	9,12 -Octadecadienoic	$C_{18}H_{32}O_2$
C18:3ω6	γ-Linolenic	6,9,12-Octadecatrienoic	$C_{18}H_{30}O_2$
C18:3ω3	α-Linolenic	9,12,15 -Octadecadienoic	$C_{18}H_{30}O_2$
C20:0	Arachidic	Eicosanoic	$C_{20}H_{40}O_2$
C20:1	Gadoleic	Eicosenoic	$C_{20}H_{38}O_2$
C20:2		Eicosadienoic	$C_{20}H_{36}O_2$
C20:3ω3		Eicosatrienoic	$C_{20}H_{34}O_2$
C20:3ω6	Dihomo-γ-Linolenic	8,11,14-Eicosatrienoic	$C_{20}H_{34}O_2$
C20:4ω6	Arachidonic	5,8,11,14-Eicosatetraenoic	$C_{20}H_{32}O_2$
C20:5ω3	Clupanodonic (EPA)	5,8,11,14,17-Eicosapentaenoic	$C_{20}H_{30}O_2$
C21:0			$C_{21}H_{42}O_2$
C22:0	Behenic	Docosanoic	$C_{22}H_{44}O_2$
C22:1ω9	Erucic	Docosenoic	$C_{22}H_{42}O_2$
C22:2	Brassic	Docosadienoic	$C_{22}H_{40}O_2$
C22:6ω3	DHA	4,7,10,13,16,19-Decosahexaenoic	$C_{22}H_{32}O_2$
C23:0			$C_{23}H_{46}O_2$
C24:0	Lignoceric	Tetracosanoic	$C_{24}H_{48}O_2$
C24:1	Nervonic	Cis-Tetracosenoic	$C_{24}H_{46}O_2$

Fats contain high saturated fatty acids compared to oils and are solid at room temperature [30]. The recovered or waste bio-oils (or liquids) range from C6 to C26 [16]. As indicated in Table 2.2, the predominant fatty acids present in the waste oils are in the C16 to C18 range and are MUFA, while the vegetable based oils tend to be higher in the C16:0, C18:0, C18:1 and C18:2 range [16, 32]. Compared to vegetable oils, waste oils have higher unsaturated fatty acids than saturated fatty acids [32, 33].

Table 2.2: Fatty acid composition of bio-oil feed stock by wt. % (modified from [16])

	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0 C22:0	C20:1 C22:1	SFA	MUFA	PUFA
Yellow Grease	23	1	10	**50**	15	-	-	34	51	15
Tallow	25-30	2-3	21-26	**39-42**	2	0.4-1	0.3	49-60	41-45	2
Lard	25-30	2-5	12-16	**41-51**	4-22	-	2-3	38-46	45-59	4-22
Palm	32-47	-	1-6	**40-52**	2-11	-	-	34-59	40-52	2-11
Corn	1-2	1-4	30-50	**34-56**	-	0-2	-	39-64	35-60	
Sunflower	6.0	-	4.2	18.7	**69.3**	1.4	-	11.6	19	69.6
Soybean	7-11	0-1	3-6	22-34	**50-60**	5-10	-	15-27	22-35	52-70
Rapeseed	2-5	0.2	1-2	10-15	10-20	0.9	50-60	4-7	60-75	15-30

2.1.2 Fuel properties and composition

Viscosity is a critical factor for the performance of an engine for the flow of oil through flow lines, orifices and injector nozzles [24]. As viscosity increases; pump pressures increase, fuel atomization reduces and incomplete or early combustion can occur due to high line pressure [29]. Viscosity increases with the number of carbon atoms and decreases with unsaturated bonds, thus, waste oils have higher viscosities than vegetable oils.

Density affects the break up of fuels injected in engine cylinders [29]. The compression ratio, amount of fuel injected in terms of mass (for the same fuel volume of input) and the diameter

of the fuel droplets increase as the density increases, directly impacting injection timing and injection spray patterns. Heating value as well as the engine exhaust emissions are also functions of density. Higher density fuels decrease atomization and mixing, and are associated with higher particulate matter and NOx emissions than low density fuels. Density increases with the number of carbon atoms and the degree of saturation. Waste oils have higher densities than vegetable oils due to higher SFA content.

The flash point indicates the presence of highly volatile and flammable substances in fuels [24]. Bio-oils generally have higher flash points compared to mineral diesel oils, while flash points of animal fats are higher than vegetable oils, due to higher SFA. Fuels with high flash points have lower volatilities causing delayed ignition and difficulties in engine start up.

The degree/level of oxidation of bio-oils indicates to the quality of the fuel. Fatty acids in bio-oils are oxidized to form products such as peroxides, which is represented by a gain in mass [8]. Due to the high autolytic activity and the presence of unsaturated fatty acids and PUFA in waste oils, lipids can be subjected to lipolysis (enzymatic hydrolysis) and oxidation [34]. Several different indicators are used to measure oxidation, where FFA content is a basic indicator. Peroxide value measures primary oxidation products, and "anisidine value" is a measure of secondary products. Total oxidation or totox value combines ansidine and peroxide values [35].

Thermal properties such as the melting/freezing temperature, crystallization, enthalpy and specific heat capacity, and reactions such as thermal oxidation and decomposition impact fuel properties. Negative melting/freezing points and high thermal stability is desired in a

fuel. Thermal stability measures the changes in weight due to thermal oxidation and/or decomposition [8]. Constituents of bio-oils such as; TAG, water and other impurities, aging and amount of heat treatment the oil was subjected to, affect freezing and melting points [36]. High levels of SFA (30 – 60 wt. %), as found in waste oils result in above $0^{o}C$ melting or freezing points. Below 0 ^{o}C melting points occur when unsaturated fatty acids are present, which further decrease with the number of double bonds [8, 36].

Pour point, cloud point, cold filter plugging point (CFPP) as well as freezing point are critical in determining the cold temperature performance of a fuel. Pour point defines the lowest temperature at which a fuel can be pumped or poured at which a gel appears [29]. Cloud point measures the temperature at which a wax appears. Fats and oils derived from biological feed stock generally tend to have low cold temperature properties. Bio-oils from animal fats and greases have poor cold flow properties compared to vegetable oils, due to higher SFA content [29, 30]. Beef tallow, for example, has about 50 wt. % SFA, making cloud and pour points occur at higher temperatures than vegetable oils [30].

The cetane number correlates to both the specific gravity and the distillation temperatures of diesel fuels [24]. Fuels with high cetane numbers such as diesel oil causes lower ignition delays and particulate emissions. The cetane number increases with chain length and level of saturation, and decreases with branching [29]. Bio-oils derived from greases and animal fats have higher cetane numbers.

Relative effect of fatty acid classes on the fuel properties is summarized in Table 2.3. The table indicates that Freezing point, oxidation stability and cetane number increases with the

saturated fatty acid content and NOx emissions decrease, while the opposite occurs with the increase in the PUFA content. Therefore, fuels that have higher MUFA are preferred due to better overall properties than fuels with higher SFA and PUFA [16].

Table 2.3: Relative effect of fatty acid classes on fuel properties [16]

	Higher SFA	Higher MUFA	Higher PUFA
Freezing point	High	Moderate	Low
Oxidation stability	High	Moderate	Low
Cetane Number	High	Moderate	Low
NOx emissions	Low	Moderate	High

2.2 Methods of Recovery/conversion

Bio-oils are recovered from biomass mainly using extraction processes, however, bio-chemical processes such as fermentation, digestion and hydrolysis are also used [18, 37].

2.2.1 Pyrolysis

Pyrolysis of biomass involves the thermal breakdown at high temperatures (350 - 700 °C) in the absence of oxygen and in some cases, in the presence of a catalyst [24, 30, 38]. Products of biomass pyrolysis include; bio-gas, bio-char and bio-oil, depending on the pyrolysis system used [19]. Pyrolyzed gas is predominantly CO, CO_2 and methane (CH_4); bio-oil is composed of organic acids, esters, alcohols, ketones, aldehydes, phenols, alkenes, nitrogen compounds, furans, guaiacols, syringols, sugars, miscellaneous oxygenates and inorganic metals, while char is predominantly elemental carbon and hydrogen [38]. The degree of pretreatment and operating parameters determines the distribution of products and depending on the type of pyrolysis (conventional/slow, fast or flash) a higher solid or oil fraction is produced [38, 39]. Conventional pyrolysis uses low temperatures (400 °C) and higher residence times (higher than 20 s), while fast pyrolysis uses moderate temperatures (500 °C)

and shorter retention times (1 s) [40]. Fast pyrolysis also produces higher oil fractions (75 wt. %), compared to conventional pyrolysis. The biomass pyrolysis process is shown in Figure 2.2 where the biomass is dried to less than 10% moisture, ground (2 mm for fluidized bed reactors), the pretreated feed is cracked in the reactor, and the three products (gas, oil and char) are further separated. Rapid heating rates and residence times of a few seconds or less are used in flash pyrolysis but, require that the particle size of feed is reduced to 105 – 250 μm [38].

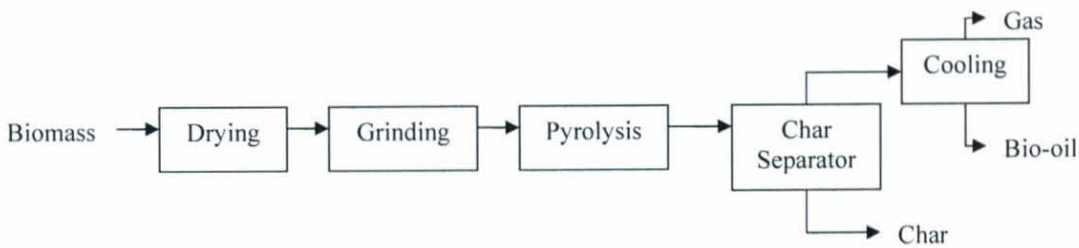


Figure 2.2: Fast pyrolysis of biomass feedstock [38]

Rapeseed cake, sunflower presses bagasse, hazelnut shells, cottonseed cake and safflower seeds have been studied using both conventional and fast pyrolysis [38]. The study by Chaala *et al.* (2003) has used pelletized beef and bonemeal flour to produce 35.1 wt. % of bio-oil through vacuum pyrolysis (conventional pyrolysis under a vacuum) [41]. Additionally, the product comprised of gas, solid and aqueous phases. Adebanjo *et al.* (2005) pyrolyzed lard at 600 °C, and produced a bio-oil similar in heating value, Cetane number, and specific gravity to that of petroleum diesel [42]. According to the same study, Green Oasis Enviro Economics Inc. used pyrolysis followed by distillation of tallow to obtain bio-oil with a distillation curve, flash point (60 °C) and pour point (-28 °C) similar to diesel.

2.2.2 Biological conversion processes

Fermentation and hydrolysis are biological conversion processes used for recovering oil from waste biomass. Hydrolysis is carried out by the decomposition of organic matter with the addition of water [3, 12, 35]. Fermentation uses bacterial culture and sugar or organic acids to digest organic matter (animal fats) and produce silage, where oil is recovered as a by-product [43].

2.2.3 Extraction

Oil extraction can be done through a physical process involving; homogenizing, heating, pressing and filtering as outlined in Figure 2.3. Vegetable based biomass seeds are first crushed, then pressed to squeeze the oil, and filtered [16]. In comparison, oil recovery from yellow grease may only require dewatering and filtering [21]. Mechanical separation methods using heat, filtration and centrifugation are used to remove some of the solid impurities and water from brown grease. Fats and oil from meat products are recovered through rendering (crushing and/or grinding of animal fats followed by cooking at controlled temperatures and mechanical or solvent separation) [16, 26]. Hydrolyzing is prevented during cooking/heating, water is typically removed; however this has resulted in increasing FFA content [16]. Mechanical separation of oils from the cooked fat is carried out through pressing followed by centrifuging [26]. Supercritical CO_2 extraction is also employed for extracting oil from meat products [16].

2.2.4 Purification

Extracted fats and oils still contain TAG, sterols (ST), water, free fatty acids (FFA), phospholipids (PL), proteins, waxes and gums, pigments and residual solids [44, 45].

Physical and chemical refining methods are used to remove impurities. Colour compounds, FFA, phosphatides and other minor contaminants are removed through chemical refining, where the oil is degummed, neutralized, bleached, hydrogenated and deodorized as outlined in Figure 2.3 [16, 24, 26].

Water and PL are removed through degumming, while neutralizing partially removes the FFA [36, 45]. The residual FFA, metals, aldehydes, KET, and natural pigments are removed by bleaching, where the oil is mixed with natural or activated clays. Hydrogenation can be used to improve the quality of oil. Deodorizing or stabilizing the odour is achieved through the removal of volatile compounds by vacuum steam distillation.

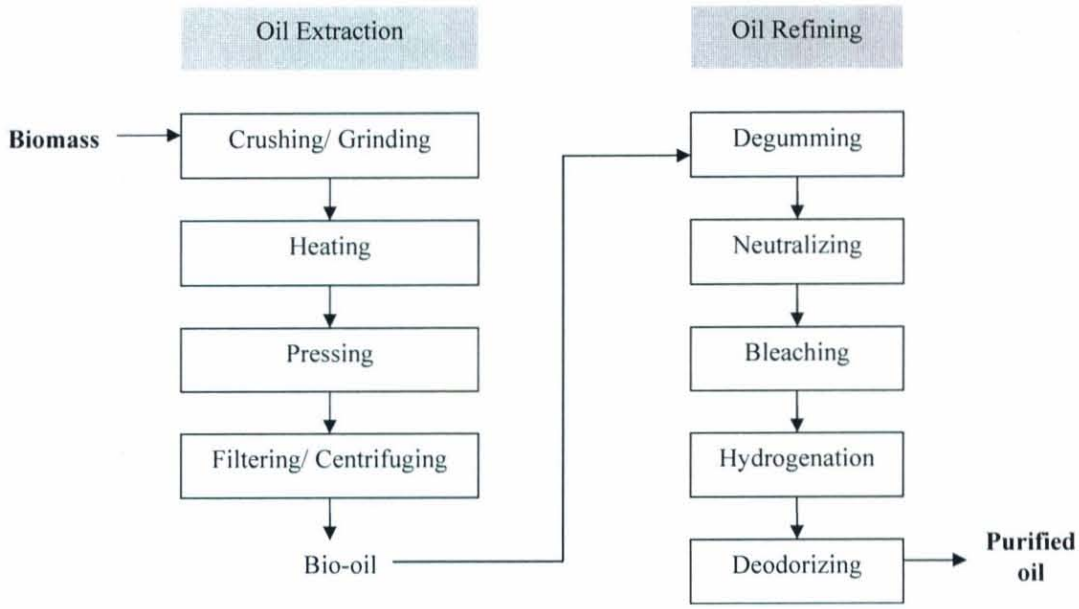


Figure 2.3: Process for recovery and chemical refining of bio-oils from biomass [16, 36, 45]

Membranes that are selective to TAG have been proposed for purification of oil and offer simplicity in use and lower cost [46 - 50]. Separation occurs through molecular size, where the molecular weight distribution of lipid classes are in the ranges of; 250 – 300 kDa for FFA, 550 – 650 kDa for diglycerides, 750 – 850 kDa for TAG, 600 – 800 kDa for PL and, greater than 950 kDa for polymer gums [50]. Membranes used in impurity removal of bio-oils are summarized in Table 2.4. The use of non porous membranes (composite, polymeric and hydrophobic) has shown to be effective in removal of several contaminants [46, 47]. Membranes with silicone as the active layer and polyimide as the support layer have removed oxidation products, polar compounds, PL, and moisture. A comparison of advantages and disadvantages of several physical and chemical bio-oil refining processes is given in Table 2.5.

Table 2.4: Membrane pore size and the selectivity of membranes [49, 51 - 53]

Purpose/ Selectivity	**Type of Membrane**	**Membrane material**	**Pore size (μm)**	**Source**
Dewaxing	Microfiltration	Synthetic polymer	0.02	[52]
Degumming	Ultrafiltration	Polyimide	20 kDa (molecular weight)	[53]
Lipid separation	Microfiltration	Polypropylene coated with mixtures of polymer solutions	0.1	[49]
Dewaxing	Microfiltration		0.2	[52]
Moisture below 0.08 wt% of animal fats	Microfiltration	Teflon (0.3 MPa pressure)	0.05	[49]
Moisture below 0.08 wt% of animal fats	Microfiltration	Polyethylene (0.3MPa pressure)	0.03	[49]
Extremely fine precipitated proteins	Microfiltration	Glass microfiber filter	0.7	[51]
PL	Nanofiltration		4 nm	[45]

Table 2.5: Advantages and disadvantages of crude bio-oil refining processes [45, 47]

Method	Advantages	Disadvantages	Source
Active filtration using absorbents	- Removes particulates - Reduced energy use	- Changes the chemistry of the oil - High capital costs - Lack of proper filtration equipment - Can leach foreign matter to the oil	[45]
Distillation	- Improves product yield - Reduced effluent volumes	- Requires higher pretreatment - High energy consumption - Can affect the stability of oil	[47]
Chemical refining (Figure 2.2)	- Removes sediments and proteins - Reduced viscosity	- High energy consumption - Pollutes the effluent streams - Large use of water and chemicals - Can destroy natural antioxidants	[47]
Membrane separation	- Retains desirable components - Low energy use - Operates at low temperature and pressure - No chemicals or solvents use - Reduces oxidation products, water and sediments, in oil simultaneously - Retains natural antioxidants in the oil	- Lower flux - Can affect oxidation stability - High capital cost	[46]

2.3 Engine performance of crude bio-oils

Crude bio-oils have a number of positive properties compared to petroleum fuels such as high heat content, lower processing requirements and renewability, however, they tend to have poor cold flow properties, high viscosity, low volatility, and high reactivity (due to high levels of unsaturated carbon chains) [54].

Direct use in engines can be difficult due to gum formation and carbon deposits due to polymerization reactions and lubricating oil thickening [37]. Engine deposits may also be attributed to incomplete combustion and partial vapourization of crude bio-oils due to their high viscosity and low volatility [55]. The composition of some oils can slow gum formation; for example, winter rapeseed oils contain up to 46.7% erucic acid content, compared to 75 - 85 wt. % linoleic acid in other oils, reducing gum formation. The PUFA in waste oils from animal fats and greases have lower oxidation stability, increasing degradation and engine

deposits [55, 56]. Even small amounts of PUFA can affect oxidation stability of bio-oils to a much larger extent than those with high SFA or MUFA [56].

Crude vegetable oil and blends were tested in engines as far back as the 1980's, where a diesel fleet was powered with a blend of used filtered cooking oil and 5% biofuel, without issues in engine filter build up or coking [57]. However, lubrication oil contamination occurred due to polymerization of PUFA, thus requiring the change of lubricating oil every 4000-5000 miles. In direct-injection engines some vegetable oils and blends showed better results than others; for example, sunflower oil blends did not show favourable results, whereas, safflower oils passed the engine manufacturer's association 200 hour test [30]. Most issues associated with direct use of bio-oils and blends occurred over long term. Coking and trumpet formation on the fuel injectors (leading to poor fuel atomization), gelling and contamination of lubricating oils, oil ring sticking and thickening, orifice plugging and, carbon deposits are some of the common issues reported in internal combustion engines [30, 55]. Preheating of fuel prior to injection, fuel switching to diesel at partial load operation, partially refining the oils to remove gums, and filtering to reduce particle size (less than 4 microns) are proposed to address some of the above mentioned issues [58].

Crude bio-oils are currently used as blends with No.2 and No.6 fuel oils in industrial boilers with minor modifications [16]. They have the capability to replace middle distillate petroleum based fuels; however, refining processes are typically employed to improve fuel properties of bio-oils for use over a wide range of applications. Refining is also necessary to reduce engine deposits, engine durability issues and lubricating oil contamination [59]. Refining processes and implications in engine use of refined biofuels are given in section 2.4.

2.4 Refining processes

The crude oil is converted to lighter products with properties similar to conventional fuels. Pyrolysis, microemulsification, hydrocracking and transesterification are the most well studied processes [30, 60]. A conventional hydro-processing has been investigated, where an iso-paraffin rich diesel substitute was produced from bio-oils [23]. Catalytic cracking and dilution are also proposed [15, 24].

2.4.1 Transesterification

Transesterification is the most commonly used method for conversion of waste and virgin bio-oils to biodiesel. In this process, Triacylglycerides are converted to fatty acid alkyl esters by replacing the alkyl group of the glycerol with hydroxyl groups using alcohols, as presented in Figure 2.4 [29]. The viscosity is reduced during the process without affecting the Cetane number and the heating value. A catalyst is used in the process (acid, base, or enzymes) and is a function of the composition of the crude bio-oil feed [30].

Base catalyzed transesterification

Base catalyzed transesterification is used for the derivation of bio-oils with FFA contents lower than 5 wt. % [15]. Figure 2.4 outlines the global reaction where the TAG in bio-oils react with alcohols in the presence of a base catalyst to form fatty acid alkyl (from the alcohol) esters and glycerol; in reality, several reactions take place, where di-glycerides and then mono-glycerides are formed as intermediate products [30].

Alcohols such as methanol, ethanol and branched chain alcohols (such as 2-propanol, 1-butanol) are common alcohols used [61]. Ethanol forms stable emulsions making separation

of esters difficult, while methanol breaks these emulsions quickly due to the presence of non-polar groups [55]. Methanol also increases the separation and quality of yield and lowers the quantity of alcohol required [16]. Methanol/oil ratios between 3:1 and 6:1 have shown higher ester production [62, 63]. Conversion efficiencies have ranged between 80% and 99.5% depending on the type of bio-oil [56, 64 - 67]. Cold temperature properties were improved with branched chain alcohols however, branched chain alcohols are higher in cost, requires higher molar ratios of alcohol/oil, and forms impurities, compared to ethanol or methanol [29, 68].

Sodium hydroxide (NaOH), potassium hydroxide (KOH), carbonates (e.g. sodium and potassium alkoxides), sodium amide, sodium hydride, potassium hydride and potassium amide are possible base catalyst candidates [30, 69]. The most commonly used base is NaOH. There, are conflicting studies on the effectiveness of sodium methoxide compared to NaOH; for example, NaOH reacted with methanol to produce a small amount of water, while sodium methoxide formed sodium salts [67, 70 - 74]. The maximum activity for the reaction requires 0.3 wt. % NaOH, while 0.5 wt. % sodium methoxide was required, therefore NaOH was better [55]. Comparison of basic alkaline-earth metal catalysts including; magnesium oxide (MgO), calcium hydroxide ($Ca(OH)_2$), barium hydroxide ($Ba(OH)_2$) and calcium methoxide as substitutes for NaOH in transesterification of rapeseed oil showed NaOH to be more effective in terms of conversion. In addition, NaOH is a low cost chemical relative to other bases [30].

The presence of water and FFA result in soap formation by partially shifting the transesterfication reaction to a saponification reaction due to the hydrolysis of TAG, thus the

feedstock and alcohols need to be anhydrous [30, 75]. Formation of soaps reduces ester yield, requires more catalyst and alcohol, results in product separation problems (ester, glycerol and wastewater), reduces catalyst efficiency, increases the viscosity of biodiesel, consumes more energy, and increases gel formation [29, 76]. This is prevented for bio-oils with higher than 5% FFA content such as in animal fats and grease by pretreatment with an acid [29, 30, 77].

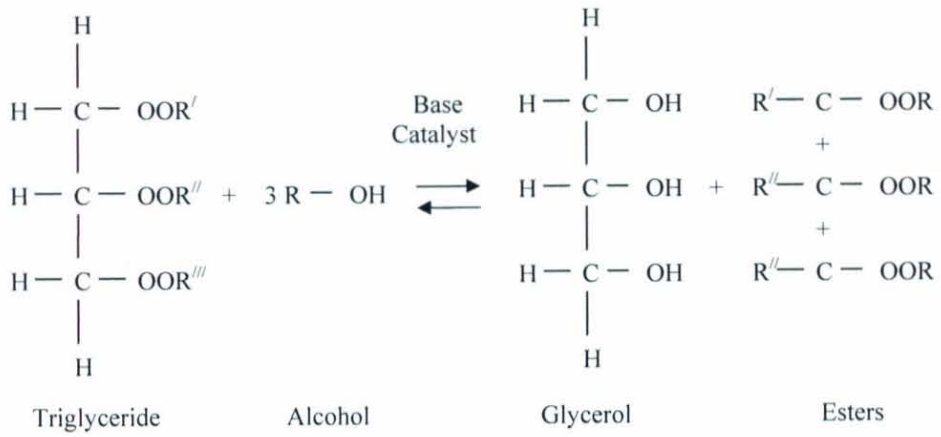


Figure 2.4: Base catalyzed transesterification reaction [29]

Acid catalyzed pretreatment and base catalyzed transesterification

Pretreatment with an acid catalyst followed by base catalyzed transesterification is used for bio-oils with FFA content greater than 5% and in some studies, greater than 0.5% [29, 55]. The pretreatment process is outlined in Figure 2.5, the FFA are converted to monoesters in the presence of the acid catalyst [29]. Organic sulfonic acid, sulphuric acid (H_2SO_4), phosphoric acid (H_3PO_4) and hydrochloric acid (HCl) are some possible acids [30]. The FFA content was reduced to about 1% in studies with yellow grease at 12% FFA and brown grease at 33% FFA [55].

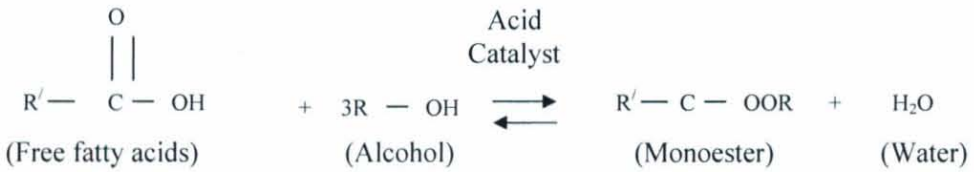


Figure 2.5: Acid catalyzed pretreatment esterification reaction

The lower dispersion of alcohol in bio-oils with high FFA (greater than 5%) will reduce reaction rates and to overcome this higher stirring rates and alcohols are used, compared to direct catalyzed transesterification [29]. Formation of water during the reaction can also inhibit further conversion of FFA to monoesters. The high alcohol use, stir rates and the need to regenerate the alcohol and catalyst result in high energy use. An alternative to the two step process is direct acid catalyzed transesterification, and here both esterifications are carried out in the presence of an acid catalyst. It does not require pretreatment for feedstock with high FFA however, use is limited due to the need for larger reactors, lower reaction rates, high alcohol use and additional corrosion control measures in reactors and engines, compared to the two step process.

Enzymatic catalysis and alternative transesterification processes

Lipases are used as catalysts in enzymatic catalyzed transesterification to achieve the same reaction as given in Figure 2.4, but with higher conversion [69]. Jackson *et al.* (1996) for example, used lipases for methanolysis of corn oil in flowing supercritical CO_2 to achieve conversion efficiency greater than 98% [78]. Lipases are able to completely convert FFA into their alkyl esters (ideal for waste oils and fats), and require minimal processing for removal of glycerol and recovery of esters [55, 69]. Lipases also catalyze transesterification in both

aqueous and non aqueous mediums [79, 80]. However, the popularization of the process is limited due to high costs associated with enzymes [69].

Supercritical methanol/ethanol without the presence of a catalyst has been studied [29, 55, 81, 82]. According to Cao *et al.* (2005), at pressure of 12.8 MPa, temperature of 280 °C, and residence time of 10 min a conversion of 98% was achieved using supercritical methanol [81]. Adding propane as a co-solvent has resulted in 100% conversion in 5 min, at 300 °C. The study by Meher *et al.* (2004) using supercritical methanol has reported complete conversion of SFA to esters at 400 °C, while unsaturates completely converted at 350 °C [55]. Pressures used during the process were not given. Simultaneous esterification of FFA and transesterification of TAG was reported in another study using supercritical methanol for feedstock with high FFA and water [29]. Use of supercritical ethanol was studied by Madras *et al.* (2004) and, 100% conversion occurred at a pressure of 2 MPa, 350 °C and a residence time of 40 min [82]. High cost due to high temperatures and pressures, high alcohol/oil molar ratios (compared to base/acid catalyzed transesterification), and thermal degradation reactions all limit application [29, 69].

Product: composition, recovery and operational issues

Products are made up of esters but also by-products that must be removed including glycerol, a mix of di, tri and monoglycerides, catalyst, and excess alcohol. Separation of the ester from the mixture is difficult due to the diglycerides and specifically monoglycerides, as mono-glycerides can cause turbidity in the product mixtures [30, 61]. Further mono and diglycerides augment hydrolysis of the ester in the presence of water, natural pro-oxidants (e.g. air), and high temperatures [24]. Glycerol can be separated through gravity separation or

centrifuging and sold for commercial use, and the remaining catalyst, soap, salts, methanol and free glycerol are removed by water washing followed by removal of water through a flash vacuum process or distillation [30, 69]. Transesterification is typically carried out at near ambient temperatures, as increasing the temperature has not increased ester conversion efficiency [67, 83].

Detection and measurement of the products is the key to determining optimal process conditions, conversion efficiencies, and product quality. Typically, a gas chromatography (GC) equipped either with mass spectrometry (MS) or flame ionization detectors (FID) are used for simultaneous determination of glycerol, mono, di and tri-glycerides in vegetable methyl esters [55]. GC analysis of products has given results lacking in consistency while derived products (free of hydroxyl groups) tend to give excellent peak shapes, high recoveries, and detection at low concentrations. High performance liquid chromatography (HPLC) have been used in product analysis which reduces analysis time, however, solvents are required as eluents. Combining HPLC with pulsed amperometric has been used for detection of free glycerol in vegetable oil esters with higher accuracy and the detection of residual alcohols was also possible [84, 85]. Other HPLC systems used include, reverse phase HPLC with ultra violet detection, evaporative light scattering detection, or atmospheric pressure chemical ionization MS in the determination of products from transesterfication of rapeseed oil [86]. Isocratic liquid chromatography with a density detector and solvents have been used for detection of mono, di, and tri-glyceride based methyl esters with good results [87]. Gel Permeation Chromatography with a refractive index detector was used for analyzing mono, di, and tri-glycerides, alcohol, glycerol and methyl esters, and sample preparation involved only dilution and neutralization [88]. The balance is choosing analytical

system(s) that can detect/measure a range of products from the reaction with low costs and ease of sample preparation.

2.4.2 Pyrolysis

Pyrolysis is used for converting vegetable and waste oils to lighter products. Pyrolysis of TAG form; alkanes and alkenes (60%), and alkadienes, aromatics and carboxylic acids (9 – 16%) [30, 69, 89]. Pyrolysis combined with metallic salt catalysts has been successful in producing paraffins and olefins similar to petroleum based diesel fuels, by using vegetable oils [30, 90]. Soybean oil has been pyrolyzed to a product with 73 – 77 wt. % HC and safflower oils to 80 – 88 wt. % HC in other studies [30]. Other feedstock that have been tested include waste fish oil which was fast pyrolyzed after aerobic treatment and centrifugal separation [89]. Challenges in the pyrolysis of waste oils include the high energy intensity and equipment cost and the reduction in oxygenate of the final product [30]. The conversion rates for the pyrolyzed processes are not as reported.

2.4.3 Microemulsification

Microemulsification of bio-oils is carried out by adding co-solvents (dispersants) or alcohols and surfactants and forming thermodynamically stable dispersions of oil, water, surfactant and co-surfactants [24, 31]. Solvents that have an affinity to aqueous and non aqueous media are used in the process, such as methanol, ethanol, 2-butanol or other ionic or nonionic amphiphiles (compounds that have both hydrophilic and lipophillic properties) [39, 69, 91]. Conversion efficiencies and production compositions of microemulsified bio-oils are not as studied in literature.

2.4.4 Alternative processes

Shonnard *et al.* (2007) introduced the "UOP/Eni Ecofining" process, and is based on conventional hydro-processing for converting bio-oils to branched paraffin rich diesel fuel [23]. Hydro-deoxygenation, decarboxylation and hydro-isomerization reactions occur. As outlined in Figure 2.6; the process was carried out in a catalytic reactor, and mixed with hydrogen gas (H_2) at unspecified reaction temperatures. The deoxygenated liquid product is then separated from water, CO_2 and H_2, and fractionated to remove light products.

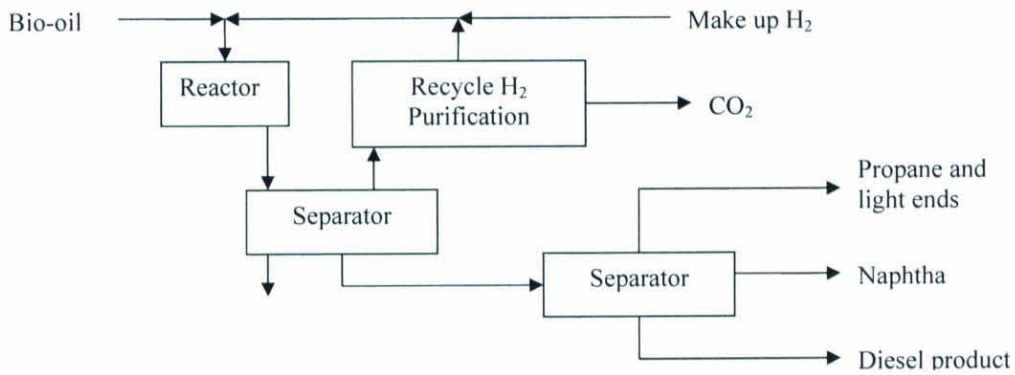


Figure 2.6: "UOP/Eni Ecofining" process [23]

Vegetable oils such as palm, rapeseed, jatropha and soybeans were tested at varying amounts of hydrogen feed to achieve 100% feed conversion of [23]. Bio-oils with high SFA, as found in recycled oils (tallow oil, fish oil and waste greases), would potentially consume less hydrogen however they would require pretreatment for salts and solids. Use of existing refineries and fuel distribution systems are possible with the process. The main product from the process is an aromatic sulphur free substance ("green diesel"), which can vary between 88 and 99 v/v% of total product. Propane and naphtha are co-products. The main product was

similar to petroleum diesel in terms of the C number range and molecular weight however; the aliphatic part was only partially saturated.

Ozone treatment was investigated by Murakami *et al.* (2004) for producing biodiesel from waste fish oil as an option to transesterfication, [92]. The process as outlined in Figure 2.7 involves pretreatment where the bio-oil is filtered using kaolin, reacting with ozone in the presence of a catalyst (primary ozone treatment), followed by filtering with zeolite, and reacting again with ozone (secondary ozone treatment).

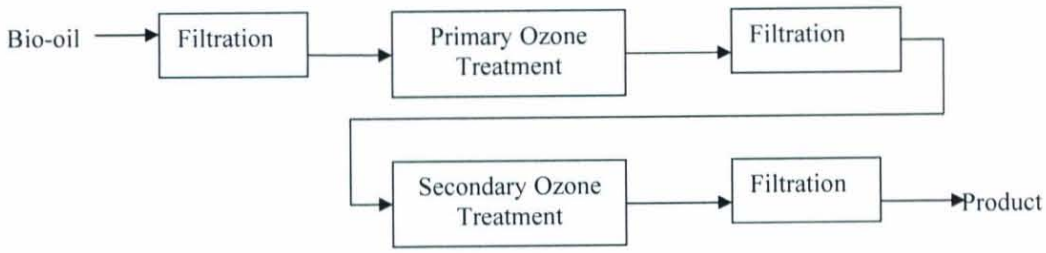


Figure 2.7: Ozone treatment of Bio-oils [92]

Ozone is decomposed in this process due to its low oxidation stability and produces active oxygen, which decomposes the bio-oil. A catalyst was added (calcium phosphate monobasic or iron oxide) to prevent polymerization of the oil during primary treatment. The process was tested for waste fish oil, and is discussed in later sections. Conversions for primary and secondary ozone treatment of fish oil were 96% and 95%, respectively. The low boiling point fractions of fish oil increased with ozone treatment, where mainly HC were present in the product and undecane was the predominant alkane [92].

Dilution of crude bio-oils with diesel fuels, solvents or ethanol is another method of improving bio-oil properties [24]. Bio-distillation, using existing petroleum processing refineries with minimal modifications, is a method under research in North America [16].

2.5 Biofuel standards

Standards have primarily, but not exclusively, focused on biodiesel. Austria was the first to prepare and approve biodiesel standards and was for rapeseed oil methyl ester [55]. Biodiesel standards have two components; general fuel based parameters similar to those of diesel fuels, and chemical composition and purity based parameters for fatty acid alkyl esters [93]. The latter is used to address biodiesel from different origins. Fuel based parameters are given in Table 2.6 and Table 2.7 summarizes the quality based parameters [55].

Iodine value (IV) is a measure of oxidation stability of a bio-oil and is based on total unsaturated fatty acids (MUFA and PUFA) and correlates with the cetane number and viscosity [56]. Iodine value is not incorporated into ASTM standards but is associated with the European Union (EU) standards. The use of IV as an indicator for oxidation stability is debated, because although higher IV values indicate lower stability, the reverse is not a given. Oils with a high level of PUFA, for example, would have high IV and therefore would indicate low stability, however, the type of unsaturated fatty acid (PUFA vs. MUFA) also impacts the stability in that high PUFA levels can further lower stability.

Table 2.6: Fuel based parameters of biodiesel [55]

Parameters	Unit	Austria (ON)	Czech Republic (CSN)	France (Journal Officel)	Germany (DIN)	Italy (UNI)	USA (ASTM)
Density at 15 ^{0}C	g/cm^3	0.85–0.89	0.87–0.89	0.87–0.89	0.875–0.89	0.86–0.90	–
Viscosity at 40^{0}C	mm^2/s	3.5–5.0	3.5–5.0	3.5–5.0	3.5–5.0	3.5–5.0	1.9–6.0
Flash point	°C	100	110	100	110	100	130
CFPP	°C	0/ -5	-5	-	0–10/ -20	-	-
Pour point	°C	–	–	-10	-	0/ -5	-
Cetane number		≥49	≥48	≥49	≥49	–	≥47
Neutralization number	mgKOH/g	≤0.8	≤0.5	≤0.5	≤0.5	≤0.5	≤0.8
Conradson carbon residue	wt. %	0.05	0.05	–	0.05	–	0.05

ON – Österreichisches Normungsinstitut

CSN – Czech Standards Institute

DIN – DIN Deutsches Institut für Normung

UNI – Ente Nazionale Italiano di Unificazione

ASTM – American Society for Testing and Materials

Table 2.7: Quality based parameters of biodiesel [55]

Parameters (m/m %)	Austria (ON)	Czech republic (CSN)	France	Germany (DIN)	Italy (UNI)	USA (ASTM)
Methanol/ethanol	≤ 0.2	-	≤ 0.1	≤ 0.3	Chapter 1 ≤ 0.2	-
Ester content	-	-	≥ 96.5	-	≥ 98	-
Monoglyceride	-	-	≤ 0.8	≤ 0.8	≤ 0.8	-
Diglyceride	-	-	≤ 0.2	≤ 0.4	≤ 0.2	-
Triglyceride	-	-	≤ 0.2	≤ 0.4	≤ 0.1	-
Free glycerol	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.05	≤ 0.02
Total glycerol	≤ 0.24	≤ 0.24	≤ 0.25	≤ 0.25	-	≤ 0.24
Iodine number	≤ 120	-	≤ 115	≤ 115	-	-

2.6 Engine performance of biofuels

2.6.1 Biodiesel

A comparison of properties of biodiesel produced from waste and virgin oils is given in Table 2.8. Conversion of crude oils to biodiesel/ester form has brought the properties close to petrodiesel. However, as the table indicates vegetable oil biodiesel, methyl soyate, ethyl

tallowate and ethyl greasate have higher viscosity, density and pour point and lower heating value than petro diesel. Conversion of crude oils to their ester form also improved lubricity and reduced premature wearing of fuel pumps [68].

Table 2.8: Properties of biodiesel produced from several feedstocks [22, 23, 32]

Property	Unit	Mahua biodiesel	Methyl Soyate	Ethyl Tallowate	Ethyl Greasate	Diesel
Density at 15^0C	kg/m^3	880	-	-	-	850
Viscosity at 40^0C	mm^2/s	3.98	4.3	5.2	6.2	2.60
Flash point	°C	208	-	-	-	68
Pour point	°C	6	-2	3	-1	-20
Water content	%	0.04	-	-	-	0.02
Ash content	%	0.01	-	-	-	0.02
Carbon residue	%	0.2	-	-	-	0.17
Acid value	mg KOH/g	0.41	-	-	-	0.35
Calorific value	MJ/kg	37	39.8	39.6	39.9	42
Stability		-	-	-	-	Good
Cloud point	°C	-	0	15	5	-

Biodiesel has low volatility and high viscosity, therefore at low temperatures it may gel and cause engine filter clogging causing problems in pumping the oil to and from engines [29, 68]. Blending it with No. 2 diesel can improve fuel properties by decreasing the pour point, cloud points, and IV. Additionally, blending improved the lubrication of ultra low sulphur petroleum diesel fuels [26, 94]. The most common blend is B20 [24].

The use of biodiesel in engines generally results in increases in nitrous oxide (N_2O) emissions, and possible crystallization below 0 °C resulting in separation of diesel from the blend and plugging of fuel lines and filters [24]. These can be improved by adding branched chain esters such as isopropyl esters and/or winterization or inducing crystallization through cooling [95, 96]. Winterization allows the residual solids to be filtered after the solution

reaches equilibrium with the esters at a cloud point below and pour point above the typical values. Saturated fats can be removed through winterization, resulting in an oil with higher long chain fatty acids [97]. Additives can also improve the pour point without impacting the cloud point [96].

2.6.2 Oil from pyrolysis

Compared to crude oil, pyrolyzed products have low viscosities and high Cetane numbers [24]. The sulphur, water, sediment content, and copper strip corrosion values are within the acceptable ranges for pyrolyzed vegetable oils, however, drawbacks include high ash content, carbon residue, and high pour point compared to diesel fuels. Waste fish oil treated with fast pyrolysis followed by distillation can result in a bio-oil boiling range similar to gasoline and diesel [89]. The use of pyrolyzed bio-oils in engines has resulted in carbon deposits and lubricating oil contamination [30]. Engine tests were mostly limited to short term durability tests [24].

2.6.3 Microemulsified oils

Microemulsions produce oils with higher latent heats of vaporization, lower viscosity, improved spray characteristics, and better ability to cool down engine chambers reducing coking, than diesel fuels; however, with lower volumetric and heating values compared to diesel fuels [24, 30]. Engine performance of microemulsified bio-oils over short term engine use is outlined in Table 2.9, and indicates to heavy carbon deposits in fuel lines [98 – 101]. Deposits in injector nozzle orifices and exhaust values in engines even during short term use have limited the use of microemulsified bio-oils [24].

Table 2.9: Results for short term testing of microemulsified vegetable oils in engines [98 - 101]

Type of feedstock	Microemulsion	Solvents	Carbon deposits	Engine performance	Source
Crude soybean oil	Ionic and non ionic	aqueous ethanol		- low Cetane number and energy content than No. 2 diesel	[98]
Alkali refined and winterized sunflower oil	Non ionic	53: 13.3: 33.7 (v/v%) of oil: ethanol: 1-butanol	Heavy carbon deposits	- No deteriorations in performance - irregular injector needle sticking - incomplete combustion - increase in lubricating oil viscosity	[99]
Degummed and alkali-refined soybean oil	Non ionic	50: 25: 5: 20 (wt. %) of No. 2 diesel: oil: ethanol: 1-butanol	in-take valves, tops of cylinder liners, injector tips	- Passed the 200hr EMA test and performed better than sunflower oil blends with diesel	[100]
soybean oil	(Not given)	52.7:13.3: 33.3:1 of oil:methanol:2-octanol:Cetane improver		- carbon deposits in injector nozzles and exhaust valves	[101]

2.7 Emission comparison of biofuels

Most studies on emissions have focused on biodiesel. Neat biodiesel (B100) can achieve emission reductions of 73% CO_2 (on a life cycle basis), 67% unburnt HC, 48% CO, 47% PM, 100% SO_2 and 80% polycyclic aromatic hydrocarbon (PAH) emissions, when compared with diesel [21]. However, there is 10% increase in NOx emissions. Lower CO_2 emissions are attributed to the presence of additional oxygen in the biodiesel. The study by USEPA (2002) performed a life cycle analysis on engine exhaust emissions using 20% blends with diesel and 100% of soybean oil, rapeseed oil, and animal fat based biodiesels. Particulate emissions and CO were reduced, while NOx emissions increased slightly in all three cases [102]. The highest overall PM and CO reduction and lowest NOx increase occurred in the 100% animal

fat based biodiesel, which was a combined feed stock of tallow, grease and lard. Shonnard *et al.* (2007) used soybean based "green diesel" and reported 83.6 - 85.2% GHG reduction over the life cycle from raw material extraction, production through to engine use, compared to petroleum diesel fuels [23]. This value was slightly lower than soybean biodiesel.

Biodiesel derived from virgin oils also had higher net energy benefit compared to most petroleum fuels [7]. Vegetable oils, for instance return 3.2 units of energy per unit of energy consumed, while petroleum diesel return only 0.83 units.

Waste oil based biofuels such as those derived from animal fats had lower life cycle based gaseous emissions than virgin oils, when the crops are harvested or produced specifically for biodiesel production [21]. Natural Resources Canada (2002) conducted a life cycle analysis study on GHG emissions of biodiesel produced from canola oil, soy oil and animal fats (a combined feedstock of yellow grease and tallow) in use as a transportation fuel [103]. Table 2.10 outlines the percentage reductions in emissions for blends of B20 and neat biodiesel (B100), compared with petroleum diesel fuels, modified from NRC (2002) data as given in WISE (2004). Animal fat based neat biodiesel shows the highest reduction in CO_2, CH_4, CO, chloroflurocarbon (CFC) + hydroflurocarbon (HFC), sulphur dioxides (SOx) and PM emissions, and the lowest increase in NOx emissions.

Table 2.10: Percentage reductions in emissions by substitution of petroleum fuels with several biodiesel products [modified from 21, 103]

	B100 biodiesel			B20 biodiesel		
	Canola	**Soy**	**Animal fat**	**Canola**	**Soy**	**Animal fat**
GHG						
CO_2	-67%	-67%	-73%	-13%	-13%	-14.2%
CH_4	-39%	-40%	-51%	-8%	-8%	-10%
N_2O	+99%	+161.7%	-1635%	+19%	+31%	-3%
Total CO_2 equiv	-64%	-63%	-92%	-12%	-12%	-18%
Non-GHG						
CFC + HFC	0%	0%	0%	0%	0%	0%
CO	-70%	-69%	-74%	-13%	-13%	-14%
NOx	+53%	+52%	+2.7%	+10%	+10%	+0.5%
VOC ozone weighted	-43%	-42%	-28%	-8%	-8%	-5%
SOx	-20%	-21%	-43%	-5%	-5%	-9%
PM	-12%	-12%	-39%	-4%	-4%	-9%

VOC – Volatile Organic Carbons

The sustainability of communities may also be positively impacted by biofuel use through possible in-community biofuel production/processing, using local materials/wastes, and use in the community itself [21]. This would result in reduction of dependency on imported energy and transportation fuels, lower the instability associated with petroleum fuel price fluctuations, creation of new expertise, economic development and overall reduction in GHG emissions and other human and environmental impacts.

Chapter 3

Literature Review – Biofuels from Fish Processing Plant Waste

3.1 Fish waste: Characteristics, composition and by-product recovery practices

3.1.1 Generation and characteristics

Waste generated from fish processing plants is approximately 50 wt. % of the harvested fish weight depending on the type of fish, product and processing techniques [45, 104]. Production for human consumption are 40% of prawns, 39% of crustaceans, 14% of mussels, 32% of crabs, 35% of brown shrimp and 35 - 45% of catfish, as a percentage of total weight of fish and the rest is disposed of. The processing varies considerably, however a "general" process used mainly in ground fish, herring and salmon processing plants in Atlantic Canada is outlined in Figure 3.1. Shell fish processing has different features to the flow given below, as lobster and shrimp processing involve butchering or cooking of live catch [1]. Processing used in fishmeal plants is discussed under section 3.3

The effluent is a result of pump water from fish unloading operations, wash water from fish cutting operations (filleting, skinning, heading, peeling, butchering of live fish), and brine water from curing [3]. Vessel unloading is carried out using wet pumps to allow smooth handling of fish, during which part of the circulating water is separated through gratings and discharged to the wastewater stream. Residual water from large wash tanks used for washing fish prior to cutting also enters the wastewater. Cutting operations are carried out with a flow of water in place, to allow for the removal of impurities. Fish parts such as skin, bones, liver, viscera, belly trimmings, gut material and kidney mixed with blood are discharged to the

effluent stream from these operations. Specific to salmanoid and pelagic fish discards are; down-graded whole fish parts, filleting by-products such as heads, belly flap trimmings and frame bones. In addition, preservatives such as phosphate, chlorine from sanitation water, chlorides, disinfectants, dockside waste, sulfates, polychlorinated biphenyl (PCB), brine water and fecal coliform from seabirds attracted to the waste also end up in the effluent stream [1, 105, 106].

Several papers have characterized the effluent in terms of solid content, biological oxygen demand (BOD), chemical oxygen demand (COD) and fats, oil and grease (FOG) [1, 4, 107]. Solids were analyzed for total, suspended, volatile and dissolved solids. The results covering a range of plants from Canada, North Carolina - USA and Egypt are given in the Table 3.1. Although constituents of the waste varied due to the type of fish, season and the processing aspects; high BOD, total suspended solids (TSS), up to 60% FOG from butchering processes and high nitrogen content due to high blood and slime, characterized the wastewater [1, 4, 105 - 107]. Fishmeal plants generated wastewater with higher organic substances and solids, than the processing plants.

The effluent can also be odorous and associated high turbidity [69]. The pH varies depending on the type of plant and typically is slightly acidic [4, 5, 44, 108, 109]. Fishmeal plant effluents had pH within the 6.3 – 6.9 range [5, 109]. Effluent from the fish cannery industry processing tuna and mussels in Spain had a pH value of 8.10, although the cooking process effluent was between 6.18 - 6.95 [44, 108]. The pH values of effluents from shell fish such as blue crab, soft shell clams and Atlantic oyster were 7.63, 7.11 and 7.15 respectively [4].

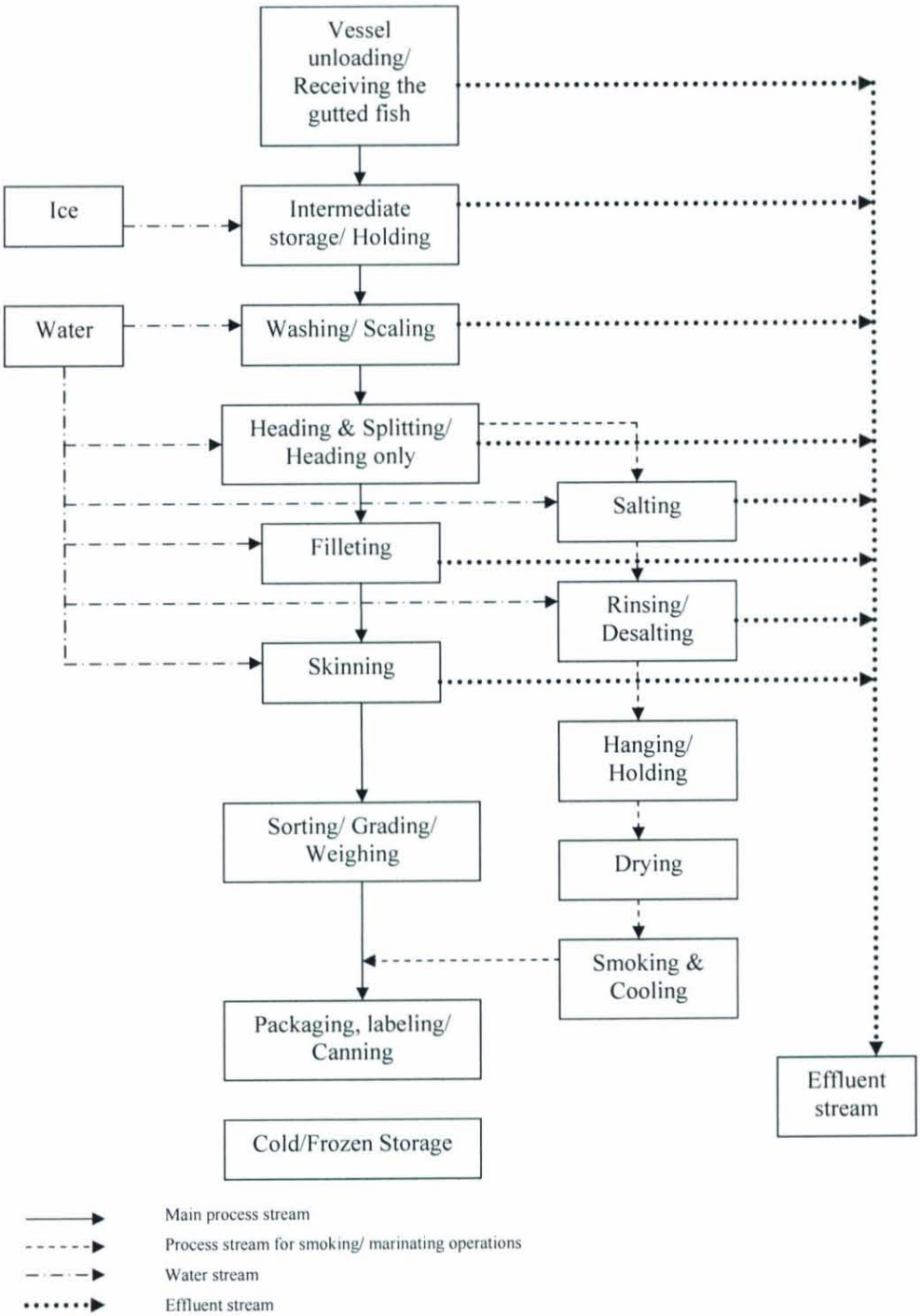


Figure 3.1: Process flow for ground fish and herring processing in Atlantic Canada [1]

Table 3.1: Fish waste characteristics from studies in Egypt, USA and Canada [1, 4, 107]

Fish/plant type	COD (mg/L)	BOD (mg/L)	TSS (mg/L)	TS (mg/L)	TDS (mg/L)	FOG (mg/L)
Tuna (NC)	1600	700	500	-	-	250
Tuna (All Canada)	-	895	1091	17900	-	-
Bottom and Fin fish (NC)	400-2000	200-1000	100-800	-	-	40-300
Fishmeal stick water (NS)	140000	48000	20000	-	-	-
Fishmeal (NC)	150-42000	100-24000	70-20000	-	-	20-5000
Fishmeal (NB)	1170 - 89800	30 - 6470	18530 - 50100	18530 - 50100	-	-
Breaded shrimp	1200	720	800	-	-	-
West coast shrimp	3300	2000	900	-		700
Sardine & Mackerel (Egypt)	1222-1431	870-1167	3745-5071	-	6217-7861	215-400
Sardine packing (All Canada)	-	100-2200	100-2100	-	-	-
Shell fish (NB)	720 - 13440	470 - 4640	180 - 5260	1080 - 22300	-	-
Ground fish (NB)	496 - 9450	180 - 4000	210 - 438	14240 - 40000	-	-
Ground fish (dry, All Canada)	-	45 – 990	14.4 – 908	-	-	-
Ground fish (wet, All Canada)	-	146 - 1205	30 - 1550	-	-	-
All Herring	3000-10000	1200-6000	600-5000	-	-	600-800
Herring (cleaning water, NB)	960 - 4800	360 – 2440	270 - 2150	264 - 1947	-	-
Herring (pickle water, NB)	64000	17920	5833	2300	-	-
Herring (NB)	-	440	500	-	-	-
Crab, Lobster, Mackerel, Herring (NB)	-	330	100	-	-	-
All Salmon (NC)	300-5500	253-2600	120-1400	-	-	20-550
Salmon (All Canada)	-	1.54 – 29.1	0.26 – 22.6	-	-	-
Snow crab, Herring (NB)	-	310	79	-	-	-
Shrimp, Crab, Herring (NB)	-	1700	1195	-	-	-

TS - Total solids, TDS – Total dissolved solids

NC – North Carolina, NS – Nova Scotia, NB – New Brunswick

3.1.2 Chemical composition

The chemical composition of the effluent depends on the harvesting region, season, type of fish, and type and extent of processing. Lipids, proteins, metals, carbohydrates and moisture are the main constituents of the discarded fish parts. Among these; lipids, volatile solids, ash and protein are important factors in determining the end use of the recovered oil as fuel [69]. Ash content is used for estimating inorganic components or metals and several papers have estimated the approximate composition [2, 10, 12, 44, 69, 104, 110]. The average composition of fish waste is given in Table 3.2. High moisture and protein were common to

all species processed [1, 3]. Ash content was generally lower than 4 wt. % for most fish waste and, lipid content varied depending on the type and part of the fish [2, 10, 69].

Table 3.2: Average chemical composition of fish waste (wt. %) [10, 12, 44, 69, 104, 111]

Type of fish	Fish part	Protein wt. %	Moisture wt. %	Ash wt. %	Lipid/oil wt. %	Source
Pink Salmon	Liver (6-16% of fish)	18.61	76.6	1.5	3.3	[10]
Walleye pollock	Liver (9-32% of fish)	7.77	41.04	0.89	50.3	
Pacific Halibut	Liver	13.36	73.31	1.3	12.04	
Pacific cod	Hand filleting	15.0	79.2	4.1	4.1	[69]
	Machine filleting	14.1	79.4	3.8	3.8	
Pollock	Hand filleting	11.3	81.3	3.6	3.6	
	Machine filleting	12.5	82.0	3.7	3.7	
Salmon	Head	14.2	71.4	3.9	3.9	
	Viscera	17.1	78.3	1.8	1.8	
Sardine & Mackerel		699-910	-	-	-	[44]
Cod	Viscera	22.1	-	1.8	74.4	[12]
Salmon	Viscera		59.4	-	24.1	[111]
Catfish	Whole viscera (33.6% of fish)	14.7	50.1	-	33.6	[104]
	Digestive tract	13.4	79.5	-	5.8	
	Liver	11.4	74.9	-	8.8	
	Gold bladder	2.6	88.9	-	0.3	
	Visceral storage (10% of fish)	1.3	8	-	90.7	
	Fillet	14.4	74.4	-	9	
	Nugget	13.5	71.2	-	14.7	

Based on the lipid content, fish are categorized into four groups; lean fish containing 2% fat (e.g. cod, haddock and pollock), low fat containing 2-4% fat (e.g. sole, halibut and red fish), medium fat containing 4-8% fat (e.g. most wild Salmon), and high fat containing 8-20% fat (e.g. herring, mackerel, farmed salmon) [111, 112]. Cod, haddock, hake, skate, ray and sharks mostly contain liver oil, while mackerel, herring and pilchard contain muscle oil [45]. A major percentage of the lipids (between 30-40%) is found in the liver of Pollock [8]. Salmon have higher fat content in the viscera than the fillet [111]. Within the same type of

fish the composition can vary depending on the type of tissue (light or dark muscle) [113]. Salmon light muscle, for example, had 20.4% protein and 2.1% fats and oils, whereas the salmon dark muscle contained 17.5% protein and 12.5% fats and oils. Marine species with red flesh generally contain higher lipid content than white flesh fish.

Metals such as arsenic, lead (Pb), mercury (Hg), and cadmium (Cd) were detected at low concentrations in waste from trout plants [2]. Phosphorous and magnesium (Mg) were 1.08 and 1.25 wt. % on a dry basis, for pink salmon livers [10]. Zinc (Zn), Mg, calcium (Ca), iorn, manganese, Cd, Pb and nickel were detected below 1 wt. % for liver discards of salmon, walleye, pollock and pacific halibut. High salinity is present in fish processing wastewater; and is attributed to ions in the fish, as well as water use and cooking during processing [44]. Tuna and mussel cooking effluents had chloride values ranging from 11.39 - 13.66 g/L [44, 108]. Seasonal average ion concentrations for sardine and mackerel effluent at a canning facility in Egypt were; 500-697 mg/L sodium ions, 60-70 mg/L potassium ions, 4.7-6.2 mg/L ammonium, 0.15-0.55 mg/L sulphate, and 13-17 mg/L phosphate [107].

3.1.3 Current practices in by-product recovery

Recovery of by-products from fish processing effluent occurs at some plants, however the majority in Atlantic Canada sends solids to landfills and wastewaters are typically discharged to the marine environment [1]. When the waste effluent is utilized it is done to produce fishmeal/oil, silage (product from fermentation of fish waste), and organic fertilizer [2].

Fishmeal plants are of particular interest as, in processing the waste from the fish plant a meal is produced for animal feed (due to the high protein and energy content) and the major

by-product of this process is waste oil [2]. Regions such as the EU, in the recent years, has imposed a ban of feeding fishmeal to ruminants (mammals who regurgitate their food as part of the digestion process, such as cows) [45]. The residual fish oil is used for a variety of purposes depending on quality, such as metal processing, leather treatment and the production of margarine, peanut butter, ink, soap, rubber, lubricants, paints, varnishes, fire retardants, fungicidal derivatives, rust inhibitors, candles, water repellents and plasticizers. Globally (in 2002) 56% of fish oil was used for aqua feed, 30% for edible oils, 12% for industrial purposes, and 2% in the pharmaceutical industry [21]. The use and processing of crude fish oil as a fuel has some history and will be discussed in subsequent sections. Fishmeal plants are not widespread, especially at remote fish locations due to high operation costs, low waste volumes associated with the fish plant itself, low value of fish waste, high cost of transportation to a central fishmeal facility (to overcome volume issues), and odour issues [114].

Other possible by-products from fish/shellfish waste include; chitan and chitosan for food preservatives, fish protein hydrolysate, fish protein concentrate, carotenoid pigment, minerals, flavours, enzymes, leather, glue, pharmaceuticals, gelatine, cosmetics, fine chemicals, collagen, pearl essence, antioxidants, and food additives [2, 45]. Separation of the "oil" from the fish waste can prove challenging as processors need to balance oil recovery with technical/operation ease and costs.

3.2 Oil recovery processes and parameters

Physical, thermal, biological processes and chemical extraction using solvents have been used at both the industrial and lab scale to recover oil from fish plant discards [20, 115].

Physical and thermal separation is used in the fishmeal industry to separate oil from the meal. Biological processes include fermentation or ensilaging and enzymatic hydrolysis [20]. In enzymatic hydrolysis the focus is on the recovery of proteins from the waste, however oil is recovered as a by-product. Solvent extraction has been used for fat/lipid extraction at the lab scale, however this may be limited in the fish processing industry due to high energy costs, requirements to build new facilities, and additional wastewater treatment requirements. The various processes are outlined below.

3.2.1 Physical/thermal Separation processes

Fishmeal process for producing oil

The process flow for fishmeal production is outlined in Figure 3.2. Effluent from processing plants is first sent for storage in pits or tanks until sufficient volumes are collected to process to fishmeal [1, 21]. The fish oil is recovered from the fishmeal plant waste by homogenizing the waste, heating or cooking it to release the oil, removal of solids by pressing, separation of oil from residual solids and water, and oil polishing [2, 7].

Hashers are used in the homogenizing step for breaking the fish into smaller parts prior to processing [7]. Laboratory scale homogenizing is carried out through mincers, grinders or blenders [35, 36, 111]. Heating, also known as "cooking", ruptures the fat cells and liberates the oil [7]. Heating also coagulates the protein and frees the physico-chemically bound water. Traditionally, heating temperatures range from 95 - 100 °C for a period of 15 – 20 min. Although fat cells start to crack at temperatures less than 50 °C, moisture content does not decrease to the optimum 10-12% until 65 - 120 °C [2]. Over-cooking can also result in the formation of large suspended solids which reduce pressability [7]. Fishmeal plants operate at

high cooking temperatures to ensure uniform temperature in heating material. Although heating conditions can depend on the type of fish, size, oil content and condition; heating at high temperatures, for long durations, may not be necessary [11]. The study by Sathivel *et al.* (2008) has added water at a ratio of 5:1 (water: ground viscera) followed by heating at 70°C for 15 min resulting in better separation of the visceral oil [36].

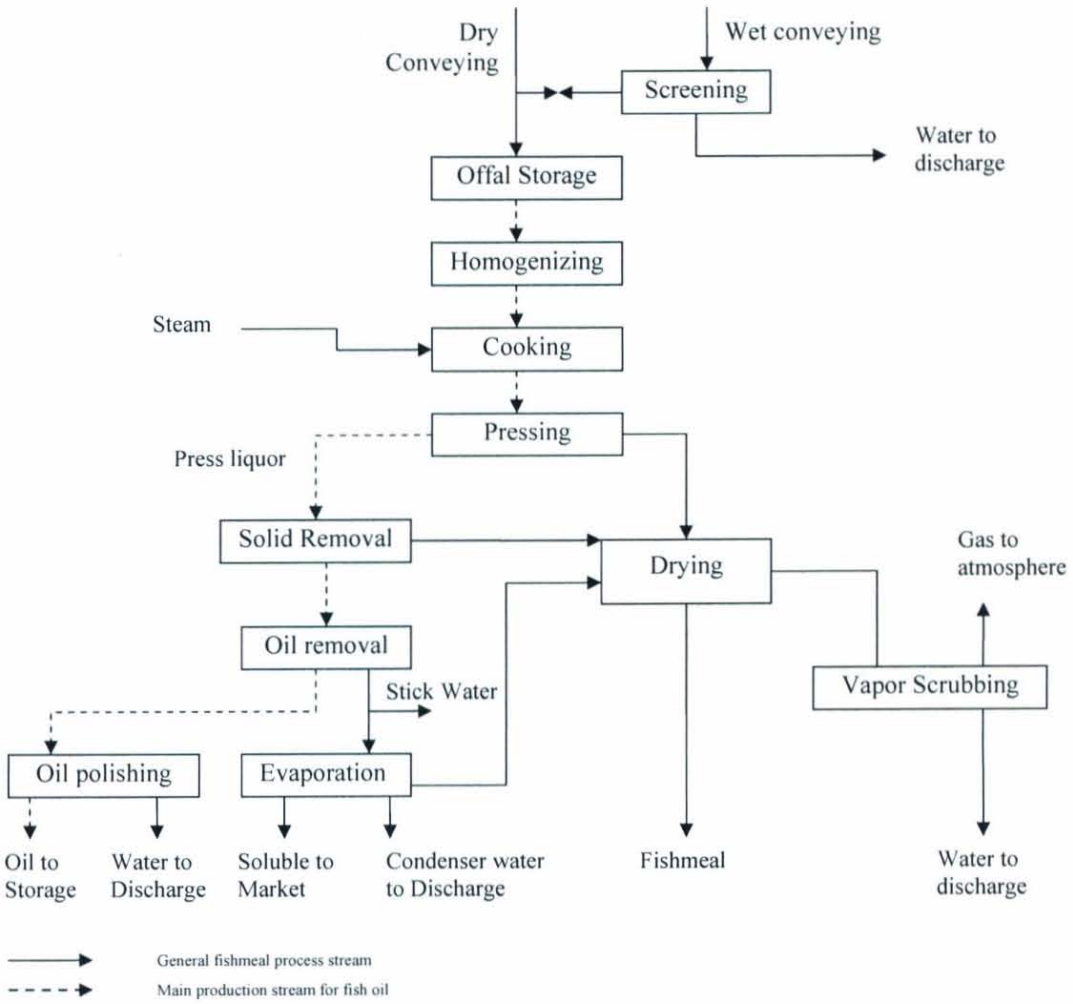


Figure 3.2: General process flow in fishmeal plants [1, 7]

Direct and indirect heating cookers are used in the fishmeal industry [7]. Indirect steam cookers are equipped with a surrounding steam heated jacket, steam heated rotor, and a rotary screw conveyer. Live steam is added to the raw material to increase the efficiency of the process. Contherm cooking is a new technology that rapidly heats the material within two minutes and provides effective temperature control, easier dismantling, and cleaning. The stick water (water removed from the effluent) is added to the fish waste in cooking by some applications, to improve the the heat transfer rate and reduce the viscosity of the raw material. Some plants use pre-cookers to reduce the load and prevent scaling in the main cookers. Direct steaming and vacuum cooking are also used to extract liver oil [45].

Pressing is carried out to squeeze liquid from the slurry and to increase the yield of the meal. Heating during pressing removes much of the moisture from the press cake, and at higher temperatures the oil viscosity is reduced but at the expense of protein denaturation and reduced oil release. Screw presses are sometimes used in conjunction with chemicals such as formaldehyde or calcium chloride ($CaCl_2$); however this reduces the nutritional quality of the oil and increases the residual chloride in the meal. The solids and liquids can also be separated using decanters (centrifuge) that can replace the press [7, 35]. The advantages of using centrifuges are; simplification of the process, better process control, reductions in heat load on the material due to a faster process, ability to process soft and very fluid like material, and better washing. Disadvantages are that the products have comparably higher moisture content and the formation of emulsions and fine particles making oil separation difficult [7].

The press liquor has oil, water, sludge and solids in both suspended and dissolved form [7]. On average 70% of the raw material ends up as press liquor while the rest is press cake. The liquor is sent to settling tanks where sludge collects at the bottom, oil on top, and the water layer in the middle. Centrifuges are used to enhance the efficiency in settling where the liquor is preheated to 90 - 95 °C prior to centrifuging. Suspended solids are removed using decanter centrifuges or desludgers [7]. The processing capacity of available decanters is 12 to 300 t/d, therefore smaller plants can use vibrating strainers to achieve the same purpose.

Vertical disc centrifuges of the nozzle type or self cleaning centrifuges are used for separation of stick water from the oil [7]. The study by Miyashita *et al.* (2004) has used a desludger at lab scale to separate the oil from liquid, where simultaneous separation of the sludge, water and oily phase was carried out using two consecutive three phase centrifuges operating at 7370 x g and 9940 x g [11]. The oil phase is dewatered using a high speed centrifuge operating at a speed of 15500 x g, at temperatures between 10 and 17 °C. Sathivel *et al.* (2008) used filtration, pressing using a cheese cloth, followed by centrifugation at 500 rpm for 30 min, for separating crude fish oil from water and residual removal [36].

The final step in preparation of oil is polishing to extract impurities and also to facilitate oil stability during storage [7]. Hot water is mixed with the oil feed at 95 °C and the oil/water mix is typically centrifuged at 5000 rpm.

Oil is recovered, generally, using a typical fishmeal process with minor modifications in heating and oil separation conditions, at both commercial and lab scale.

Other process: Lab and commercial scale

Other processes produce oil as a by-product. A large amount of water is added in rendering and fat walls are hydrolyzed by steam under pressure, until the fish waste is partially liquefied; and the oil is recovered through skimming or centrifugation [21]. The study by Miyashita *et al.* (2004) has separated sardine oil from the surimi effluent sludge using two consecutive three-phase centrifuges operating at high speeds [11]. Further separation of TAG was carried out by passing the oil through a column packed with n-hexane and diethyl ether at a ratio of 9:1. The oil recovered through this process can be used commercially for food grade applications without further refining.

3.2.2 Extraction using chemicals

Solvent extraction and acid based digestion are the two most common methods to separate oil at the laboratory scale. These processes are focused on separating the lipid fraction with minimum impurities for chromatographic analysis in of fatty acids and lipid classes. These experiments have used various mixtures including methylene chloride, chloroform/methanol (2:1), chloroform/methanol/chloroform and methanol 2:1 mix/chloroform extracted water (2:1:1:0.5) for extracting oil from wastewater or fish bodies [11, 28, 35]. Acid digestion experiments carried out by Sun *et al.* (2006) has used concentrated HCl with tertiary butylhydroquinone (TBHQ) as an anti-oxidant for extracting lipids for fatty acid analysis [111].

3.2.3 Biological processes: Enzymatic hydrolysis and Fermentation

Hydrolysis

Enzymatic hydrolysis is used for recovering proteins from effluents in the fish processing industry where fish oil is a by-product [12]. Autolytic hydrolysis enzymes occurs by using enzymes (fish viscera are rich in these enzymes such as lipases) present in the fish [20, 43, 116-118]. However, this dependency on digestive enzymes and long reactor residence times may reduce the quality of the hydrolysates (compounds produced by hydrolysis) [12, 119]. Raw materials with high lipid content can form protein-lipid emulsions during autolytic hydrolysis and reduce the oil yield [12, 120, 121]. Enzymes are added in accelerated hydrolysis and the temperature controlled to enhance protein production.

Eperiments by Ivar *et al.* (2005) has investigated the effect of heating/temperature, initial water addition and addition of two types of enzymes (Lecitase and Alcalase) on the product quality of cod by-products [12]. Initial heating increased the percentage oil recovered up to a high value of 51% and lowered protein-emulsion formation. However, the TAG production was reduced and impurities such as; PL and polar lipids increased. It was found that using lecitase as the enzyme reduced lipid degradation during hydrolysis [12, 122]. The highest oil production and lowest emulsion formation was achieved with Alcalase enzyme without the addition of water.

Supercritical and sub-critical water hydrolysis has been investigated in several studies for recovery of oil from fish waste [9, 113, 123]. According to Yoshida *et al.* (1999), subcritical processes showed higher oil recoverability from fish waste than the supercritical process, including the production of other useful organic materials such as pyroglutamic acid and

amino acids (cysteine, glycine and alanine) [123]. Yoshida *et al.* (2003) separated subcritical water hydrolyzed product compounds using an ion-exchange column [113].

Fermentation

Fermentation of fish waste is carried out by using bacteria and sugar or organic acids (eg. lactic acid) where silage is produced and the oil is typically separated by centrifugation [20]. Acids generated in-situ from microorganisms have also been used in fermentation of fish viscera where the bacteria oxidized the fat as well as preserve it [20, 124-128]. Silage preparation from fish viscera through bacterial addition carried out by Rai *et al.* (2010) showed the pH value of fresh viscera reduced from 6.1 to 4.5 after three days of fermentation, where the higher acid values is attributed to bacterial lipases and delay in the fermentation process [20]. Significant differences in oil product were not observed between fermenting under natural conditions and controlled conditions, making the former method better. Advantages of fermentation/hydrolysis over physical separation methods are possible energy savings and recovery of other useful products such as protein hydrolysate and collagen.

3.2.4 Refining

Washing is typically required if the crude fish oil is used as an animal or fish feed [45]. Miyashita *et al.* (2004) used solvent extraction to remove tocopherols and pigments with a slurry mixture of n-hexane, activated carbon, and celite 545 [11]. The oil, in this case, was further refined on a silicic (silicagel 60) acid column by washing with n-hexane and a mixture of n-hexane/diethyl ether solution (95:5, 90:10 and 80:20 v/v). A generalized

chemical refining process flow for impurity removal is outlined in Figure 3.3 and is used to remove FFA, odour, waxes and water from fish oil [8, 36].

3.2.5 Preservation of feedstock and the recovered oil

The presence of high levels of protein, fats and enzymes in fish waste can cause enzymatic hydrolysis and microbial degradation, lowering the quality of the recoverable oil [3]. High moisture levels also contribute to hydrolysis. Enzymes such as lipases in the gut material of high fat species such as salmanoids and pelagic, result in the formation of FFA. The recovered oil can also be highly reactive and degrade easily, due to high levels of unsaturation and PUFA in fish oils. Degradation of oil can form FFA and other oxidation products as described under section 3.4.7. Oxidation of unsaturated fatty acids present in fish waste lipids result in bad odours [20]. Preservation of the waste and recovered oil is essential to prevent all of the above conditions.

Preservation of the waste is achieved through chilling or icing, freezing, ensilaging with acids and freezing in combination with addition of antioxidants [3]. Use of ensilaging to preserve the material is not desirable, as the addition of formic acid results in lipid oxidation and hydrolysis. Ensilaging also increases FFA and the volume of the waste, thereby increasing the cost of transportation and further processing. Chilling or icing is effective for short term storage, but increases the bulk of the material and the energy requirements. Chilling is comparably advantageous since facilities are already available in the processing plants. Freezing can be costly due to requirement of space unless facilities are already present onsite. Salting needs further investigation in terms of biodiesel applications. Freezing in

combination with addition of antioxidants is recommended as a preservation method for fish waste.

Preservation of the recovered oil through storage in bulk tanks results in moisture collection at the bottom and partial solidification of the oil during cold weather conditions causing difficulties in pumping [3]. The use of mild steel tanks with water drainage facilities at the bottom, steam heated coils to prevent saturated oil from precipitating during winter months, and good insulation mitigates these problems. Addition of synthetic anti-oxidants to crude or refined oils is another method to minimize lipid oxidation.

3.3 Chemical and physical properties of the oil and analysis methods

3.3.1 Lipid composition

Marine oils contain TAG of fatty acids with carbon atoms varying between 14 and 22, with multiple double bonds possible. Other lipids such as FFA, waxes, ethers, HC, PL, KET, and alcohols can also be present [3, 36]. Lipid analysis is carried out through extraction with solvents such as chloroform and methanol and additional solvent development followed by thin layer chromatography (TLC) equipped with FID [28]. Hexane, diethyl ether, formic acid, acetone, methanol, chloroform solvent development systems are used [12, 28]. Lipid composition of fish processing waste extracted using several methods are summarized in Table 3.3. Analysis of ozone treated fish oil by Murakami *et al.* (2004) showed that the oil contains aldehydes, KET and ramify chain alkanes in small quantities [92]. The chain length of fatty acids in TAG of the feed (fish oil) reduced through ozone treatment, including diglycerides and formed HC. The predominant HC in the ozone treated product were alkanes with a carbon number between 9 and 14.

Table 3.3: Lipid composition of oil recovered from fish waste [10 - 12, 20, 35, 113]

Raw material	Lipid recovery method	Analysis method	TAG (wt. %)	FFA (wt. %)	Other (wt. %)	Total lipid recovery	Source
Catfish Viscera	Solvent extraction	AOAC procedure 985.14	-	-	-	-	[35]
Walleye pollock	Solvent extraction	-	92.15%	3.28%	-	-	[10]
Pacific halibut	Solvent extraction	-	89.94%	1.87%	-	-	[10]
sardine by-products	Solvent extraction	TLC/FID	>99%	-	-	-	[11]
Whole sardine	Solvent extraction	TLC/FID	85%	1%	13.5% PL	-	[11]
fish by-products	Enzymatic hydrolysis	-	65 - 89%	0.2 - 1.2%	3% cholesterol, 7.6% PL and polar lipids	87-97%	[12]
sardine by-products	Centrifugal Separation	TLC/FID	>99%	-	-	-	[11]
Whole fish	Sub-critical water hydrolysis	-	-	-	-	0.2 - 64%	[113]
Fish viscera	Fermentation	-	-	-	-	75 – 85%	[20]

3.3.2 Fatty acid composition

Fish oil contains long chain PUFA (n-3 or ω-3), the prominent ones being EPA and DHA making it an attractive edible oil [11, 111]. Cold water marine fish contain a large amount of ω-3 fatty acids with Atlantic herring, Atlantic salmon, rainbow trout and sardines having the highest ω-3 fatty acids, while the lowest is in catfish [10, 129]. Additionally, 50 different types of fatty acids can be present in fish oils [20, 113]. The fatty acid composition is determined using a three step analysis is carried out; extraction of the fat, derivatization to their alkyl ester form and analysis [11, 20, 92]. The derivatized fatty acids are analyzed using GC/FID.

The study by Godiganur *et al.* (2010) has carried out a quantitative and qualitative analysis of crude commercial fish oil by GC showed a fatty acid profile of 24.8% stearic, 23.6% palmitic, 9.84% myristic and 6.56% octadecatetraenoic acids [130]. A study by Bechtel *et al.* (2006) has shown fatty acids for Alaskan fish livers (pink salmon, walleye pollock and pacific halibut) were between C14:0 and C24:1, while seventeen types of fatty acids were detected for the farmed Atlantic salmon in a study by Sun *et al.* (2006) [10, 111]. Further analyzing the PUFA composition by Sun *et al.* (2006) showed 6.99% DHA and 7.91% EPA. A study for salmon offal (viscera, whole fish, filleting by-products, heads, trimmings, skins and frame bones) by Skara *et al.* (2004) showed 9.9% EPA and 11.8% DHA in the ω-3 fatty acid (21.7% of offal) [35]. A summary of fatty acid composition values for several fish by-products are given in Table 3.4. Catfish visceral parts showed high levels of unsaturated fatty acids in a study by Sathivel *et al.* (2002) [104]. The breakdown in this study showed unsaturated fatty acids were 307.6 mg/g for nuggets, 261.3 mg/g for whole viscera, 259.3 mg/g for visceral storage fat, 102.1 mg/g for digestive tract, 94.7 mg/g for gall bladder and 28 mg/g for liver of catfish.

Table 3.4: Fatty acid composition of several fish discards [10, 111]

	PUFA (mg/g)	MUSA (mg/g)	SFA (mg/g)	Total (mg/g)	Source
Pink Salmon Livers	376.45	269.20	113.98	759.63	[10]
Walleye Pollock Livers	204.05	343.25	207.46	754.77	[10]
Pacific Halibut Livers	215..96	334.45	143.88	694.29	[10]
Farmed Atlantic Salmon Viscera	322.20	360.50	313.70		[111]

Oil recovered from work involving fermentation or enzymatic hydrolysis of fish viscera had equal distribution of saturated and unsaturated fatty acids between C-14 and C-24 [35]. Palmitic, stearic, oleic, linoleic and linolenic acids were the major fatty acids in the oil and

the predominant fatty acid and SFA were oleic acid and palmitic acid respectively. The distribution in fresh viscera was, 1.79% EPA and 2.84% DHA in fresh viscera while the fermented product had between 1.41 - 1.74% and 2 .54 -3.02% of EPA and DHA. The amount of unsaturated fatty acids remained constant over fermentation. A study involving surimi wastewater by Miyashita *et al.* (2004) showed EPA (15.9%), DHA (11.6%) and palmitic acid (15.9%) were the main fatty acids in the recovered oil [11]. Experiments using subcritical water hydrolysis of horse mackerel waste to recover oil carried out by Terashima *et al.* (2003) contained mainly EPA and DHA [113].

The experiments by Murakami *et al.* (2004) where ozone was used to recover oil from the waste has analyzed the raw fish waste oil using GC/MS qualitatively and GC/FID quantitatively [92]. Very small quantities of EPA and DHA were detected and the main fatty acids found were myristic, palmitic, stearic, oleic and linoleic. Fatty acid composition of biodiesel produced through transesterfication of oil recovered from fish discards were analyzed by Lin *et al.* (2009) and was 20.94 wt. % oleic, 19.61 wt. % palmitic, 5.24 wt. % stearic and 15.91 wt. % DHA [131]. The percentage long chain fatty acids (C20 – C22 range) and SFA were 37.30 wt. % and 37.06 wt. % respectively, while percentage PUFA in the fish biodiesel was 28.46 wt. % compared with 0.64 wt. % in waste cooking oil based biodiesel

3.3.3 Thermal properties

Thermal properties of the waste and fish oil are critical for determining storage conditions, end uses and overall stability. Differential scanning calorimetry (DSC) is a key instrument used for analyzing thermal properties [132]. A summary of thermal properties (melting point,

enthalpy of melting and specific heats) from various studies for fish oils at different steps in the processing are given in the Table 3.5.

Table 3.5: Thermal properties of crude and purified fish oils [8, 36, 133]

Type of fish	Purification step	Melting range (°C)	Enthalpy (kJ/kg)	Specific heat capacity (kJ/ kg. °C) at 20 °C	Source
Catfish	Crude	-46.2 – 21.2	74.1	1.69	[36]
Catfish	Degummed	-45.9 – 11.5	74.7	1.96	[36]
Catfish	Neutralized	-44.3 – 11.4	75.1	1.97	[36]
Catfish	Bleached	-47.1 – 9.9	79.3	1.91	[36]
Catfish	Deodorized	-52.3 – 8	84.3	1.83	[36]
Pollock oil	Unrefined	-69.5 - 14.2	36.9	1.5 – 3.2	[8]
Red Salmon oil	Unrefined	-69.6 - -0.36	40	0.8 – 1.6	[133]
Pink salmon oil	Unrefined	-64.7 - 20.8	39	1.3 – 2.3	[133]

Table 3.5 indicates that the melting point decreased with each purification step, with the deodorized oil showing the highest melting point reduction [36]. Removal of impurities at each refining/purification step did not affect enthalpy of the melting range.

Thermogravimetric analyzer (TGA) uses the initial temperature and amount of weight losses during heating, as indicators of thermal stability [8, 134]. Weight loss is due to decomposition or interactions between compounds such as phospholipids, complex metals, minerals, FFA, peroxides and other oxidation products present in the oil. Stability is indicated by a high initial temperature of decomposition. Analysis of crude pollock oil in the TGA under an air atmosphere by Sathivel *et al.* (2008) showed large weight loss between 200 and 450 °C and weight loss increased with temperature where complete decomposition occurred at 535 °C [8]. Oxidation under an air atmosphere occurs due to the absorption of oxygen resulting in the formation of peroxides; and identified through a gain in mass as

shown in the study by Hassal (1996) [8, 134]. However, weight gains did not occur in pollock oil and the weight loss characteristics of red and pink salmon oils studied by Sathivel (2005) were similar to pollock oil [8, 133]. Thermal decomposition was higher in refined fish oils than crude fish oils due to higher availability of heat for evaporation of volatiles in refined oils, as absorption by impurities are reduced [8].

3.3.4 Rheological and cold temperature properties

Rheological properties are important parameters to determine how the fish oil/waste can be transported and handled [8]. Viscosity is the most important property as it is the key indicator of flow characteristics and is measured using rheometers. The apparent viscosity of pollock oil as measured by Sathivel *et al.* (2008) at a shear rate of 500 s^{-1} and at 20 °C was 0.04 ± 0.001 Pa.s [8]. The flow behaviour index of pollock oil in the same study ranged from 0.8 to 0.9 exhibiting non-Newtonian behaviour where the viscosity changed with the shear rate.

Viscosity, cloud and pour point of fish oil ethyl esters were tested by Wilson's fuels of Nova Scotia in their pure form and with blends with No.2 diesel fuel [68]. The ASTM D2700-91 and ASTM D97-96a were used for cloud and pour point measurements respectively. Viscosities of both neat fish biodiesel and blends (B20, B40, B60 and B80) increased with decreasing temperature across all shear rates. Both neat biodiesel and blends followed Newtonian behaviour down to their pour points, and pour points of biodiesel blends decreased with an increase of No.2 diesel in the blend. The cold temperature flow and rheological properties of fish oil generally improved over conversion to biodiesel.

3.3.5 Oxidation stability

Titration is typically used to determine FFA and peroxide values in fish oils which will in turn indicate oxidation stability [34, 35]. Fourier Transform Infrared Spectroscopy (FTIR) has been used to overcome some of the drawbacks and cumbersomeness of the titration method and has shown better precision [34, 135, 136]. Near-Infrared Spectroscopy (NIR) was also used for FFA content measurement for mackerel oil and salmon fillets. Spectrophotometry has been used for measuring ansidine values (secondary oxidation products) [35]. Saponification and conjugated diene values are used as stability indicators of fermented fish by-products [20, 35].

The storage temperature, time and atmosphere affect lipid oxidation and hydrolysis of fish oil [3, 35]. A study by Skara *et al.* (2004) has shown storage at 4 °C and in nitrogen environments inhibits oxidation, as indicated by lower change in ansidine, peroxide and totox values when compared to higher temperatures and oxygen environments [35]. Peroxide formation at lower temperatures is related to lipid and antioxidant concentration while at higher temperatures it is due to oxygen [137]. Sathivel *et al.* (2008) studied the effect of time and determined accumulation of peroxides was increased after 10 weeks of storage at 24 °C [8]. A study of salmon skin stored over 120 days showed a linear increase in FFA content from 0.6% to 4.5% due to autoxidation [34]. Lipid oxidation of oils recovered from fish liver over storage temperature and time can be higher than other parts due to the active enzymes present [46]. Overall, studies indicate lipid oxidation increased with storage time and temperature; however other components in the oil mix may inhibit this effect. Skara *et al.* (2004) for instance studied the oxidation of salmon oils over 60 days, in air at 23 °C and did not find high levels of oxidation [35]. This is attributed to the antioxidant effect by the

caretonoid content or astaxanthin pigments of the oil leading to higher stability [35, 138]. Low levels of EPA and DHA, and formation of peptides can also act as antioxidants [20].

Fermentation of visceral oil resulted in slight increase in the peroxide value with no significant change in conjugated diene value [20]. However, there was hydrolysis of lipids to FFA due to lipases present. Fish biodiesel produced through transesterification of marine fish discards as studied by Lin *et al.* (2009) had higher initial oxidation stability than waste cooking oil biodiesel or commercial biodiesel, due to higher SFA in fish biodiesel reducing peroxide formation [131]. The study also showed that fish biodiesel had lower stability than commercial biodiesel with time due to the higher PUFA in fish biodiesel.

3.3.6 Other physical properties (water content, colour and density)

Physical properties such as colour, water content and specific gravity/density have been studied by several authors [8, 12, 111, 133, 139]. Chemically refined pollock oil detected with a Minolta Chromameter in a study by Sathivel *et al.* (2008), was "light yellow" [8]. Similarly, Chaala *et al.* (2003) found hydrolysis of fish waste resulted in clear yellow coloured oil [41]. Crude oil from Japanese fishmeal plants was black in colour [11].

Bulk density values in studies were 0.902 ± 0.004 g/mL, 0.9 g/mL, 0.81 g/mL and 0.911 g/mL for pollock, red salmon, pink salmon and herring oils, respectively [8, 133, 139]. Water content and water activity are measured using aqua lab water activity meters and microwave moisture analyzers. Sathivel *et al.* (2008) determined the water activity of pollock, red salmon and pink salmon oils and the results were 0.464, 0.57 and 0.53 respectively [133].

Overall, fish oils have higher viscosity, lower lubricity, more acidity and higher flash point, compared to petroleum diesel. The main concerns raised from earlier research for engine use of fish oils were engine deposits in exhaust ducts and increased wear in parts which are constantly in contact with the oil [14]. These are discussed in later sections.

3.4 Fish biofuel as a fuel

3.4.1 Crude fish oil

Crude fish oil in diesel engines

The UNISEA pollock oil demonstration project used crude fish oil as a blend with No.2 low sulphur diesel oil in 2.3 MW medium speed two cycle stationary engine generator sets, using pure diesel, 50%, and 100% fish oil blends [14]. Emissions and engine durability were conducted and compared to 100% diesel use. The oil was a by-product from fishmeal plants processing the fish waste. Properties of the oil are compared with other studies as given in Table 3.6. Heat and sulphur contents were generally lower than for diesel fuels across all blends.

Crude fish oil in internal combustion engines

Fairbanks Morse Engine Division of Coltec Industries Inc. has tested crude fish oil and its blends with diesel in a medium speed, two cycle, six cylinder engine [140]. Testing was carried out with fish oil, derived from pollock oil in surimi plants, as an alternative to diesel fuel for electricity generation. Fish oil blends were varied between 0% and 100%, at 10% increments in the blend with diesel and tested for performance characteristics, emissions, combustion cycle characteristics, and deposits/wear in engine components. Load conditions of 50%, 75% and 100% were used. Properties of the 100% fish oil are given in Table 3.6.

Compared to the blends the pure fish oil had lower volatility. Thermal cracking of the fish oil and blends occurred and the onset temperature of cracking decreased with increase of fish oil in the blend. When compared with 100% diesel, the initial boiling point of all fish oil blends was higher, but pure fish oil boiling point was lower than all blends and pure diesel. The Calculated Cetane Index for measuring ignition delay was not applicable to the fish oil as thermal cracking occurred prior to the mid boiling point. The Calculated Carbon Atomic Index as developed by Shell Oil Co. for testing of heavy petroleum fuels proved to be a better method, and the values of fish oil blends and diesel were similar. The lubricity tests carried out using "A Ball on Three Seats" test showed an increase in wear of the fish oil by a magnitude of 1000 compared with 0.3% diesel fuel.

Crude fish oil in combustors/furnaces

Wang *et al.* (2004), on behalf of CANMET, carried out a study using Alaskan derived crude fish oil, for combustion characteristics of the oil such as emissions and flame stability [13]. Several blends with No.2 and No.6 fuel oils were tested for use in boilers and furnaces for heat and/or power generation. Properties of the used crude fish oil are compared in Table 3.6. The crude fish oil had higher H:C ratios compared to No.6 fuel oil. Flash and pour points were much higher than both fuel oils; however, the calorific value was slightly lower. The kinematic viscosity of fish oil was much lower than No.6 fuel oil at all temperatures tested, as was the viscosity of blends. The lower viscosity of fish oil reduces the preheating requirements and pump demand of the blended oil. Density, calorific value, kinematic viscosity and C, H, N and ash contents of the fish oil were similar to No.2 fuel oil.

Table 3.6: Comparison of fish oil properties from several studies [13, 14, 140]

Properties/ Study	Unit	UNISEA	COLTEC	CANMET
Density at 15^{0}C	(kg/m^3)	922.5	923.7	876.9
Calorific value	(MJ/kg)	39.4	37.4	40.0
Flash point	(^{o}F)	230	331	293
Pour point	(^{o}C)	-	-9.4	3
Cloud point	(^{o}C)	-	-	3.2
Specific Gravity		-	-	0.876
Acid Number	mg KOH/g	-	8.63	-
Kinematic viscosity	(cSt)	-	33 (at 38^{o}C) 22.4 (at 50^{o}C)	5.42 (at 25 ^{o}C) 4.18 (at 40 ^{o}C)
Initial boiling point	(^{o}F)	-	322	-
Cracking Temperature	(^{o}F)	-	567	-
Moisture	(wt. %)	-	-	0.05
C	(wt. %)	-	78.92	77.21
H	(wt. %)	-	11.59	12.08
S	(wt. %)	0.0084	0.004	0.0034
N	(wt. %)	-	-	0.003
O	(wt. %)	-	-	10.39
Ash	(wt. %)	-	0.0032	<0.001

S – Sulphur

O - Oxygen

3.4.2 Fish biodiesel

Ocean Nutrition, a division of Clearwater Fine Foods, has been producing biodiesel from residual fish oils since 1999 in Nova Scotia [141]. The residual oil is a by-product from ω-3 dietary supplement and functional food production from ω-3 fish oils. The oil is purchased from large scale processors in South America. Part of the produced oil is used for their own energy needs in the boiler and heating system while the rest is distributed through local subcontractors. Biodiesel (fish oil methyl ester) production is 1million L/y. Biodiesel (fish oil ethyl ester) is also sold by Wilson's fuels for use as home heating oil and/or for blending with transportation fuels [68].

Aquafinca in Honduras operates a plant where biodiesel is produced from fish oil separated from tilapia waste [129]. About 11,000 L of oil is separated on a daily basis from 100 t of

tilapia waste, and the plant produces 6000 L of biodiesel from the recovered oil. This translates to producing 1.13 L of biodiesel/ kg of fish waste. The oil recovery process is made up of cooking in a boiler and heating to 100 ^{o}C, separation of the liquid from the biomass using an expeller, and recovery of the oil from water and residual solids using pre-clarifier. The separated oil is heated, reacted with caustic soda and methanol (20%), and decanted to separate the oil from glycerine to produce biodiesel. The biodiesel is purified through decanting, washing with water vapour at 95 ^{o}C, drying, further purification and filtering, prior to sending for storage or pumping/filling stations. The properties of the recovered oil or biodiesel are not given in the study. A life cycle based environmental impact assessment is not still conducted for the process. Issues to be addressed include; markets for glycerin, easy access points for obtaining the fish waste, and reliable sources of purchasing methanol and caustic soda.

Steigers Corporation converted about 45,425 L of fish oil obtained from processors at-sea to 37,854 L of biodiesel with 93% conversion efficiency [142]. Properties of the produced biodiesel were compared with ASTM D6751 biodiesel standards as given in Table 3.7. About 132 million litres of fish oil are produced in the Alaskan Aleutian Isands (Unalaska, Dutch harbour), however due to high costs associated with transporting it to other markets this had limited commercial value [143]. A study was conducted in Taiwan by Lin *et al.* (2009) on oil recovered in the fishmeal industry for biodiesel production [131]. A mix of waste from mackerel, salmon, tuna and cod fish processing was used as the raw material, and the oil was separated by cooking it in boiling water, squeezing the liquid, and centrifugation. The recovered oil was brown in colour and contained water, fish residue, saline compounds and other impurities. The oil was refined through absorption using active clay, winterizing at

4 °C, centrifuging, water washing, and finally heating to 105 °C. Transesterification was carried out by using sodium methoxide as the catalyst, and adding methanol at a 6:1 ratio (fish oil: methanol). The reaction mixture was homogenized at constant temperature and glycerol separated through gravity settling. The crude biodiesel was distilled to remove the unreacted methanol, and further washed with 50 wt. % petroleum ether, followed by distilled water. The lipid content of the crude fish oil was 37 wt. % and increased to 85 wt. % after refining and 92 wt. % after transesterification. Therefore, biodiesel conversion efficiency was 28.9%, and the energy output/input ratio was 5.35. Properties of the biodiesel are compared in Table 3.7.

A similar study conducted in Taiwan by Lin *et al.* (2009), used discarded parts such as; viscera, gills, fins and heads of mackerel, herring and cod for production of biodiesel [144]. The fish oil was obtained by squeezing the fish discards and removing impurities, water and soap, stirring, winterizing (for removal of impurities and components), water washing and centrifuging. The transesterification process used methanol at a 1:6 ratio (fish oil:methanol), and 1 wt. % NaOH as the catalyst. Glycerin was removed through gravity settling and centrifuging. Water washing and heating was used to remove unreacted catalyst, methanol, water, volatile compounds and any other impurities. Biodiesel obtained in neat form was tested and the results compared with No.2 ASTM diesel and commercial biodiesel from waste cooking oil. The resulting properties are summarized in Table 3.7.

A study conducted in India by Godiganur *et al.* (2010) has analyzed engine performance and emission characteristics of fish biodiesel and its blends. The produced fish biodiesel was a transparent light yellow colour and without any suspended matter [130]. The biodiesel was

obtained from manufacturers and suppliers of biodiesel (karanja and mahua oil) to TATA motors and the process details are not given. Properties of the fish biodiesel are compared to other fuels in Table 3.7.

Other developments in biodiesel production from fish waste include ENERFISH project in Finland in partnership with Vietnam, plans for production of biodiesel from fish fat recovered from wastewater by the National Technological Centre for Canning of Fish Products in Spain, and a feasibility study conducted by the Sustainable Community Enterprises in Vancouver for converting fish oil to biodiesel [129]. While the feasibility study conducted by the Sustainable Community Enterprises (Vancouver) in 2005 showed biodiesel from fish oil is not economically viable, the 2007 study proposed a base transesterification system or a fully automated acid/base two stage systems equipped with a water wash as viable.

There are small differences between the fish biodiesel properties due to fish feedstock and processing parameters among other factors. However, most properties of marine biodiesel are close to the values of No.2 diesel. The high elemental oxygen content and slightly lower carbon residue associated with fish oils translates to better combustion properties and lower particle emissions. A potential drawback, particularly in cold climates, is the high viscosities associated with pure biodiesel and blends, when compared to diesel. Compared to the crude fish oil properties discussed in earlier sections, improvements are seen in the oil after conversion to a biodiesel.

Table 3.7: Comparison of fish biodiesel with biodiesel standards [130, 131, 142, 144]

Fuel properties	Unit	ASTM Biodiesel	ASTM No. 2 Diesel	Steigers (April 2004)	Steigers (April 2005)	Karnataka India	Taiwan
Specific gravity			0.83	-	-	0.88	0.86
Flash point	°C	100 min	74	> 190	>130	176	103
Calorific Value	MJ/kg		46.2	-	-	42.2	41.4
Water & sediment	vol. %	0.050 max	-	0.40	0.00	-	-
Carbon residue	wt. %	0.05 max	1.57	0.05	0.022	-	0.76
Sulfated ash	wt. %	0.020 max		0.021	0.000	-	
Viscosity at 40°C	cSt	1.9 - 6.5	3.4	4.4	4.3	4.0	7.2
Cetane	-	40 min	53.2	49.4	51.9	-	50.9
Cloud point	°C	3 °C	-	-1.0	-1.0	-	-
Copper corrosion	-	3B max	-	1B	1B	-	-
Total acid number	mg KOH/ g	0.80 max	-	0.14	0.39	-	1.17
Free glycerin	wt. %	0.02 max	-	0.001	0.006	-	-
Total glycerin	wt. %	0.24 max	-	0.19	0.167	-	-
Distillation	Atmos. Equiv. 90% recovered	360 °C max	-	379 °C	380 °C	-	-
P	wt. %	0.001 max	-	0.0057	0.000	-	-
O	wt. %	-	0	-	-	10.9	7.19
C	wt. %	-	87.17	-	-	-	80.01
H	wt. %	-	12.76	-	-	-	12.75
N	wt. %	-	0.07	-	-	-	0.05
S	wt. %	0.05 max		0.00023	0.00010	-	

3.4.3 Biogas from fish waste

Biogas is derived from fish oil or the waste itself and typically produced by anaerobic digestion [145]. A system used to process rainbow trout effluent consisted of a digester connected to a sedimentation column with an aerobic filter, and final polishing using a Zeolite column. The digester reduced the total, soluble, suspended, and volatile solids and the zeolite column reduced total nitrogen and wastewater contaminants.

Salmon hatchery sludge containing; 1.5-3.3% solids, 32% N, 8.5% P and low heavy metals was treated through anaerobic digestion to produce biogas [146]. The low ash and high

moisture content of fish waste proved beneficial in biogas production from fish waste [69]. Biogas was produced from the waste of fish oil/fishmeal, fish filleting, herring cannery, mackerel cannery, shell fish and smoked fish industries by using both anaerobic digestion and codigestion [147]. Reactor failure occurred at loading rates of 2g VS/L/day and higher, when co-digesting with fish and wood wastes, due to the high level of long chain fatty acids in the fish waste [148]. Results obtained from other biogas studies are summarized in Table 3.8. Other factors inhibiting biogas production from fish waste include the presence of high concentrations of different ions and high levels of sodium in the waste [69, 149].

Table 3.8: Process parameters and products of biogas from fish waste [69, 150 - 155]

Source	Seafood	Reactor	Product	Other gasses
[150]	Blue crab cooking waste water	Anaerobic digestion	6.6 – 10 L/L of feed, CH_4 – 68%	CO_2 – 28% and H_2S – 1.5%
[151]	Crab residues	Leach bed reactor + hybrid sludge-bed filter	>70% of product was biogas	
[152]	Fish offal with cattle slurry	Batch codigestion	47.3% - 31.1% volatile solids reduction	
[153]	Canning sardines and tuna	Upflow anaerobic sludge blanket	CH_4 – 0.23 L/g	
[154]	Tuna processing liquid effluent	Anaerobic cylindrical fixed bed reactor	0.25 m^3/kg COD	
[155]	Fishmeal processing effluent	Anaerobic filter	0.05 – 2 L/g COD	

3.4.4 Fish biofuels from other processes

Properties of the ozone treated fish oil in the study by Murakami *et al.* (2004) are summarized in Table 3.9. The product was compared to biodiesel produced via transesterification using a methanol and NaOH system where by-products included 10 wt. % glycerin and 50 wt. % wastewater [92]. The ozone process also resulted in a better quality

biodiesel with fewer odours, no glycerine, less waste and was associated with lower chemical use. The ozone produced biodiesel had similar or higher heating values compared to petroleum diesel and the pour and flash point values were lower than that of petroleum diesel.

Pyrolysis has also been investigated for conversion of fish oil. The study by Meier *et al.* (2009) has used a continuous fast pyrolysis plant was used to thermally crack waste fish oil [89]. The waste fish oil was pretreated using aerobic treatment followed by centrifugal separation, and fed to the pyrolysis unit as an emulsion with water. The waste fish oil converted to 15.85% gas, 11.32% coke, 2.14% solids, and an aqueous phase, and approximately 73% of bio-oil which separated by gravity from the aqueous phase. The bio-oil consisted of a light fraction in the C4-C10 range, a heavy fraction in the C11-C22 range and an oily sludge, and was distilled under atmospheric conditions to obtain purified products in boiling ranges of gasoline and diesel. Properties of the bio-oil fractions are compared in Table 3.9. Pyrolysis and distillation resulted in a reduction of density, 56% sulphur and 26.7% IV (due reduction in unsaturated HC) when compared to waste fish oil. However, the water content (due to addition of water) and acid value (due to high carboxylic acid contents) increased. The light bio-oil was 22.63% of waste oil and 31.07% of pyrolyzed oil after distillation. Compared to the pyrolyzed bio-oil, the light bio-oil had lower density and water content, similar acid values, and higher degree of unsaturation (52.56% higher), and also lower C4-C8 and higher C9-C12 compounds than gasoline. The heavy oil product was 54.25 wt. % from the pyrolyzed oil and 39.52 wt. % from waste fish oil, and had low water content, low IV and high acid values than bio-oil. Compared with Brazilian diesel specifications the heavy bio-oil fraction had higher CFPP and density and a lower cetane index. The heavy oil

also had a lower volatility and higher C4-C12 and C20-C22 contents than petroleum diesel. Both light and heavy bio-oil fractions had lower sulphur values.

Table 3.9: Properties of fish biofuels produced by ozone treatment and pyrolysis [89, 92]

Property	Unit	Ozone treated fish oil	Pyrolyzed fish oil		
			Bio-oil	Light bio-oil	Heavy bio-oil
Water content	wt.%	-	0.68	0.06	0.04
Sulphur content	wt.%	-	0.01	0.02	0.01
Soot	wt.%	0	-	-	-
Acid index	mg KOH/g	-	131.1	107.3	142.5
Iodine Index	cg I_2/g	-	64	98	67
Cold filter plugging point	°C	-	-	-	14
Distillation test Initial boiling point	°C	-	-	-	-
Kinematic viscosity at 40°C	cSt	131	-	-	4.8
Flash point	°C	38	-	-	31.5
Pour point	°C	-16	-	-	-
Density	kg/m^3	870	896.5	835.9	886.6
High heating value	MJ/kg	44.8	-	-	-
Cetane Index		-	-	-	-

3.5 Engine performance and emissions of fish biofuels

3.5.1 Engine performance of crude fish oil

The UNISEA demonstration project has reported wearing in fuel injector or engine-mounted fuel pumps, and hard deposits in exhaust component (piston ring seating grooves, exhaust ports, exhaust turbine inlet rings) in the stationary diesel engine [14]. However, higher than normal suspended and in-soluble protein content in the fish oil has increased the load on fuel purifiers and filters. Start-up of engines was better than with diesel fuel and no difficulties occurred in engine shut downs. An increase in the engine's fuel rack position and engine-mounted fuel filter pressure differentials were observed due to lower thermal content and higher viscosity. The engine's crankcase lubricating oil was tested at twenty four hour intervals and did not show contamination or unusual consumption rates. Adverse effects

during storage at cold winter conditions were prevented by re-circulating the fish oil using a heater-equipped centrifugal fuel purifier. There are limits to the application of the test results of the study due to the rare engine design used. Suggestions made for improvement are inline blending as opposed to batch blending and pre-purification of fish oil before use by the installation of dedicated fish oil centrifugal fuel purifiers and/or suitable filtration equipment. The latter is to avoid entrained water and suspended non-soluble protein from creating undesirable conditions in diesel engines.

Engine performance tests conducted in the Coltec study, for the medium speed internal combustion engine showed an increase in specific fuel consumption with increasing fish oil in the blend, across all load settings [140]. This was attributed to lower heating value of fish oil. At 100% load setting the thermal efficiency increased with increased fish oil in the blend. Combustion cycle analysis tests did not show differences between ignition delay characteristics of fish oil blends and neat diesel. Combustion pressure and heat release were also not different. Inspection of engine components after thirty seven hours of operation showed increased levels of deposits on the upper piston section, which was higher than the lower piston section. This difference is attributed to cooler surfaces of the upper piston compared with the lower piston. During the short duration of testing, severe deposits or wear was not reported. Transesterification of the crude oil is proposed to reduce deposits. Overall, except for the slight increase in deposits, fish oil and blends have demonstrated similar ignition qualities and excellent combustion characteristics compared to diesel fuel.

Testing for use as furnace oil in the CANMET study suggests fish oil as a better substitute for No.2 fuel oil due to the ability to create similar thermal effects [13]. Fish oil and 5% and

10% fish oil blends in two types of oil fired residential boilers (30 kW and 150 kW) were carried out with no adverse effects. Higher viscosities are not a concern for these applications; therefore, straight run fish oil can be used in combustors without any further refining or transesterification, greatly reducing the cost. Suggestion for better use of the oil is removal of impurities such as proteins, waxes and water that cause adverse conditions in engines and during storage.

3.5.2 Engine performance of fish biodiesel and blends

Halifax Regional Municipality Metro transit bus system tested fish biodiesel or fish oil ethyl esters between November 2003 and March 2004 [68]. Fish biodiesel blends (with petroleum diesel) of B20 in Metrobus transit fleets and B100 in municipal buildings are currently promoted by the municipality. However, use of B100 was difficult due to clogging of engine filters, issues associated with pumping from fuel tanks to engines and burners and, gelling at temperatures of 3 ^{o}C or less.

Steigers Corporation tested fish biodiesel, B100, in a generator and, B20 and B40 splash blends in utility vehicles [142]. Excellent efficiency and operability as well as material compatibility with generator engines were reported. The blend, B100 was tested for forty eight hours in a test bed engine or generator. Favourable efficiency and operability impacts and lubricity gains were observed, however, injector deposits occurred. Protein fouling occurred in engine fuel filters as well as injector deposits, which was attributed to presence of protein in feed stock. Filtering of fish biodiesel is proposed to reduce deposits.

Fish biodiesel (fish oil methyl ester) was tested in Karnataka, India in a four stroke, three cylinder air cooled direct injection diesel engine at a constant speed and varying load conditions [130]. Fish biodiesel blends of 0, 10, 20, 40, 60 and 80 v/v% with neat diesel were used to analyze engine performance in terms of brake specific fuel consumption, brake specific energy consumption, thermal efficiency and exhaust gas temperature. The observations are summarized in Table 3.10. Brake specific fuel and energy consumption and exhaust gas temperature values increased with the increase of fish biodiesel in the blend, while the thermal efficiency decreased.

Fish biodiesel was tested in Taiwan in a direct injection, four-cylinder, four-stroke diesel engine at constant torque and speeds varying from 800 - 2000 rpm [144]. The presence of PUFA in fish biodiesel improved cold temperature properties such as CFPP and fluidity, thus improving the issues with sticking, freezing and stalling of vehicles. Increases in brake power output and exhaust gas temperature occurred for B100. Higher oxygen content in fish biodiesel resulted in lower exhaust gas temperature and shorter combustion duration times compared with diesel. The fuel consumption rate of fish biodiesel was higher than No.2 diesel and lower than waste cooking biodiesel, to achieve the same energy output. Brake fuel conversion efficiency (engine power output: heat release rate ratio) was higher for fish biodiesel than No.2 diesel at engine speeds less than 1400 rpm, however lower beyond this speed, and is attributed to higher oxygen content. The "oxygen-rich advantage" decreased with increase in mixing extent in the combustion chamber. Equivalence ratio measured in terms of residual oxygen in the exhaust gas was lower for fish biodiesel than No.2 diesel.

Table 3.10: Engine performance results of fish biodiesel [130]

Property	Observations
Brake specific fuel consumption	- Increased with percentage fish oil in blend, due to lower calorific values of biodiesel than petroleum diesel - Higher for 100% biodiesel than 100% diesel - Higher mass injections for biodiesel blends than diesel, for the same volume and pressure of injection (due to higher density of blends than diesel)
Brake specific energy consumption	- Minimal value obtained for B20 and lower than that of diesel - Higher for B40, B60 and B80 than diesel due to lower calorific values
Thermal efficiency	- Maximum attained for B20, higher than that of diesel
Exhaust gas temperature	- Increased with biodiesel percentage in blend - Higher in biodiesel blends than diesel, due to higher heat loss in blends)

Fish biodiesel was tested in other studies in a single cylinder diesel engine and achieved a maximum power output 3% higher than using neat diesel [156]. Lubricity gains were reported in another study when used in blends, indicating to the potential use of fish oil blends as an additive [21]. Fish biodiesel produced through ozone treatment was tested in a Japanese automobile (103 kW at 3200 min^{-1}) without any engine modification. Compared to vegetable biodiesel, ozone treated fish biodiesel had better fuel properties [92]. A three hour engine test conducted at full loads for horse power outputs of 40%, 60% and 100%, did not indicate to engine issues. Generally, for use of low cost liquid biofuels such as waste oils/biodiesels, heavy duty medium speed diesel engines were better [130].

3.6 Emissions from fish biofuels

The in-use emission reduction by substitution of fish biofuels and blends with No.2 low sulphur diesel fuel and No.2 fuel oil in stationary diesel engines, furnaces and boilers are summarized in Table 3.11.

Table 3.11: Percentage reductions in-use emissions in engines

Source	**[13]**	**[13]**	**[14]**	
Type of engine	**Furnace**	**Residential boiler**	**Stationary diesel engine**	
Petroleum fuel	No.2 fuel oil	No.2 fuel oil	No.2 low sulphur diesel	
Blend	50% fish oil	5% fish oil	50% fish oil	50% fish oil
CO_2	4%	0%	5%	5%
CO	-6%	2%	-18%	2%
PM	-	-	43%	35%
SO_2	-40%	-4%	-40%	-40%
NOx	(NO) 11%	-1%	6%	4%
	100% fish oil	100% fish oil	100% fish oil	100% fish oil
CO_2	4%	-	6%	6%
CO	-25%	-	-28%	2%
PM	-	-	63%	43%
SO_2	-96%	-	-80%	-80%
NOx	14%	-	7%	6%

Emissions were measured in the UNISEA fish oil demonstration project for CO, NOx, CO_2, and PM and, SO_2 emissions were estimated from the fuel sulphur content [14]. Overall, maximum reductions were in pure fish oil use, and were up to 63% of PM, 28% of CO and 80% of SO_2 emissions. There were slight increases (<6%) in both NOx and CO_2 emissions. The main concern for the environment in which the powerhouse operated was SO_2, thus operating with fish oil blends was more beneficial even with the increment in NOx emissions. The study conducted by Coltec showed slight increases in NOx and CO_2 emissions with increasing fish oil in blend when compared to pure No.2 fuel oil use [13]. The residential boiler study showed an increase in CO emissions, while other emission types decreased. The study indicates that other than NO (in furnace) and NOx (in residential boiler) emissions all other emission types from pure fish oil use are lower than pure No. 6 fuel oil use. Increase in NOx emissions with the increase of fish oil in the blends is due to lower volatility of the fish oil and formation due to nitrogen in the air [13].

Ozone treated fish biodiesel showed negligible SO_2, and lower soot and particulate emissions in Japanese automobiles compared to petroleum diesel [92]. This is due to lower molecular weight fractions and higher oxygen content in fish oil, when compared with petroleum diesel. Overall, improvements in fish biofuel properties are needed for substitution of No.2 diesel oil. However, crude fish oil can better replace combustion fuels without any engine modifications. Reductions in CO_2, CO, SO_2, PM emissions are observed, but NOx emissions increased when used in their pure form.

3.7 Economics: Newfoundland and Labrador as a case

The fish processing industry is an important part of rural and remote communities in Atlantic Canada and generates approximately 418,000 t of waste per year [2]. Fish waste is generated annually in the amounts of 35,000 t, in NL alone. Four primary types of fish are commercially processed; ground fish (cod, flatfishes, Greenland product), pelagic/fin fish (capelin and herring), shell fish (shrimp, crab, and clams/quauhaug) and miscellaneous (lumpfish, seal etc.) [1]. The number of facilities in operation registered under the act by 2002 were 131 facilities; five of which were aquaculture facilities.

Fishmeal plants (2 sites) and seal processing plants (1 site) are required by the provincial law to obtain a certificate of approval under the environmental protection act. Screening is the most common type of treatment, where most gravity settled solids are collected for disposal or fishmeal processing. Discharge of fish offal at sea and land waste sites are approved for NL processors due to remote location and unavailability of facilities for fish oil or meal processing. However, regulations may become stringent in the future requiring better waste management practices.

Minimal by-product recovery is done in NL due to remoteness, high costs and high moisture content [3]. Capelin, mackerel, farmed salmanoid, seal blubber and herring discards are either used as bait or as animal feed in certain plants. Some capelin and seal blubber discards are also sold to foreign food grade markets. With only a few fishmeal plants in operation, oil recovery is minimal. Oil recoverability of fish discards of certain species processed in NL were estimated by a study for Environment Canada and are given in Table 3.12. Fat content by weight of species and assumed percentage recoverability of 80% were the basis for the estimation, and varied between 3 to 23%. This translated to about 965 t/y of oil in the waste at an 80% recovery. However, only 5% of herring oil is currently recovered.

Table 3.12: Fat content and recoverability of fish discards of NL [3]

Type of Fish discards	Year	Estimated fish processing discards (kg)	Fat content (wt. %)	Assumed oil recoverability (kg)
Capeline	2005	3,372,100	3.80	96,105
Herring	2004	8,335,680	9.04	565,158
Mackerel	2004	2,362,724	13.89	246,136
Seal Blubber	2005	8,999,301	87.70	7,892,387
Farmed Salmanoids (Atlantic and Steelhead)	2005	205,670	23.00	155,147

High FFA content, heterogeneity of the waste and remoteness mean processing to recover edible oils, or recovery of oil for export as a biofuel would likely not be feasible. On site processing and use as a fuel blend for applications requiring low quality oils would likely create economic benefits due to energy savings and reduced costs in disposal. Several possible applications for the fish oil are use in the plant's boiler or furnace, residential heating oil in the community or even fish processing vessels.

Chapter 4

Experimental Methods

4.1 Overview

Characterization of the fish processing plant effluent was the first step in the experimental approach. Effluent samples were from three different fish processing plants in NL operated by Nature Sea Farm Ltd, located in St. Alban's (Bay d'Espoir area). The type of waste, time of sampling, and method of preparation for analysis and storage conditions of the waste samples are given in Table 4.1. The initial testing was for the whole fish waste of cod and salmon, and showed negligible oil recovery. Therefore salmon gut material containing effluents were tested in the second stage due to the high oil content and availability of the waste. A more refined analysis was carried out for a third set of fresh salmon salmon gut material including the testing for storage conditions.

Table 4.1: Description of the fish processing effluent samples

Effluent sample	Type of fish discard	Time of obtainment	Temperature of storage before preparation	Preparation	Temperature of storage after preparation	Storage period before analysis
FW1 - C	Cod whole fish	August 2008	1 - 4 °C	Ground at medium speed in a lab blender for 5 – 10 min	1 - 4 °C	4 months – 1 year
FW1 - S	Salmon whole fish	August 2008	1 - 4 °C	Ground at medium speed in a lab blender for 5 – 10 min	1 - 4 °C	4 months – 1 year
FW2 - S	Salmon (guts, belly trimming)	October 2009	1 - 4 °C	Ground at medium speed in a lab blender for 5 – 10 min	1 - 4 °C	1– 4 months
FW3 - RS	Raw Salmon (guts, belly trimmings)	January 2010	4 °C	Ground in a the Hobart grinder	-26 °C	2 weeks – 2 months
FW3 - FS	Frozen Salmon (guts, belly trimmings)	January 2010	-26 °C	Ground in a the Hobart grinder	-26 °C	2 weeks – 2 months

The Cod whole fish (FW1 – C) and fresh salmon whole fish (FW1 – S) for the first lab scale experiments and the salmon gut materials (FW2 - S) for the second stage experiments were obtained through the Department of Fisheries and Aquaculture. These were stored at 1 – 4 °C at the lab cooling storage and homogenized in a lab scale blender. The Centre for Aquaculture and Seafood Development of Marine Institute (CASD) assisted in obtaining and preparing the third set of salmon gut material (FW3) samples. Part of the waste was freshly stored at 1 – 4 °C (FW3 – RS) and the other part was frozen at -26 °C (FW3 – FS) to test the effect of storage temperature on the composition of the waste/recovered oil. Homogenizing the FW3 – RS and FW3 - RF samples was using a 5.2 kW Hobart grinder with 3 mm diameter perforated plates and carried out at the CASD.

Characterization of the waste was carried out for testing the BOD_5, acidity and TSS, TDS, TVS, TS and residual chlorine contents. The lipids were separated from the waste and lipid class and fatty acid compositions analyzed at the Ocean Sciences Centre of the Marine Institute. Experimental methods used are described in section 4.2 and was carried out for the cod whole fish (FW1 – C) and old salmon (FW2 – S), fresh frozen salmon (FW3 – FS) and fresh raw salmon (FW3 – RS) gut material containing effluents. The salmon whole fish (FW1 – S) samples were not tested due to the limited content and the inclusion of salmon waste in the next set of samples. The FW2 – S waste was rancid at the time of testing, but results are included to determine the effect of storage temperature/time on the composition.

The next stage of the experiments was recovering the oil from the waste and the procedures are described in detail in section 4.3. Initial experiments were conducted on a mixed sample of FW1- C and FW1 - S by partially removing the oil fraction using a modified fishmeal

process and membrane filtration. Effect of heating temperature, heating time, centrifuge speed and time and membrane pore size on the composition of the recovered oil was also investigated in these experiments. The results were incorporated into designing the process for recovering oil from FW2 – S, however, again effect of heating time and temperature were tested due to the samples being salmon gut material. Other modifications such as addition of water washing step and changing centrifuge speed were also tested in this stage. Oil recovery from FW3 – FS and FW3 – RS were carried out using results from the above experiments and a special type of membrane.

Recovered and/or partially purified oil was analyzed in the final stage of experiments for physical properties such density and viscosity; thermal properties such as melting point; and specific heat capacity of oils/products from FW1 (mix of FW1 – C and FW1 – S) and FW3 (FW3 – SF and FW3 – RF). Physical properties of FW2 – S was not tested as the effect of oil recovery process conditions was determined through the compositional analysis and the physical properties of salmon gut materials was tested using FW3. Specific lipid class composition and fatty acid composition were tested for recovered oil from all three sample sets (FW1 – C, FW2 – S, FW3 – FS and FW3 - RS). The procedures were similar to the waste analysis however the procedure for extraction of the lipids from the oil was modified. The moisture was only testing was conducted only for product from FW1 to investigate appropriate moisture testing methods. The sulphur content was measured for recovered oil from the fresh salmon waste as an initial assessment of fish oil and to compare with petroleum fuels. Test procedures for physical, thermal and chemical characterization of the product/oil are described in detail under section 4.4.

The residual from the waste after recovery of oil was also characterized in terms of BOD_5, TSS, TS, TVS, TDS and residual chlorine content and acidity, using methods similar to the waste, for all three sets of waste samples (FW1 – C, FW2 – S, FW3 – FS and FW3 – RS).

4.2 Characterization of the waste

The samples FW1 - C, FW2 - S, FW3 - RS and FW3 – FS, were tested for bulk density, solid composition and BOD_5. Acidity and residual chlorine tests were conducted as pre-requisites of the BOD_5 test [157]. Tests were conducted using standard methods [157 - 159].

4.2.1 Bulk density

Bulk density measurements were carried out by measuring the weight of a known volume of oil. Glass vials were cleaned, calibrated for 10 mL and 20 mL using a burette, filled with effluent and measured for weight. Equation 4.1 was used for estimating bulk density.

$$\rho_E = W_{EKV} / V_E \qquad (4.1)$$

Where, ρ_E is the bulk density of effluent samples (g/L), W_{EKV} is the weight of known volume of the effluent sample (g), and V_E is the volume of effluent sample (L)

4.2.2 Solids testing (TDS, TS, TSS, TVS)

Testing for solids were carried out using ceramic evaporating dishes and crucibles after one hour of combustion in a muffle furnace and cooling in desiccators to room temperature for an hour and weighing. Sample sizes used were 1 - 3 g and measurements were carried out for triplicates of the waste samples.

Total solids concentration

Total solids were determined after drying the samples at 103 – 105 °C [158, 159]. Samples were first transferred into the prepared crucibles, measured for initial weight, dried for one hour at 103 – 105 °C in a drying oven, cooled in a desiccator to room temperature and weighed. The cycle was repeated until the weights of samples were 5% from original. Total solids concentrations in samples were determined using Equation 4.2.

$$C_{TS} = W_{TS} \times \rho_E / W_E \qquad (4.2)$$

Where, C_{TS} is TS concentration in effluent samples (mg/L), W_{TS} is weight of final residual after drying for total solids (mg), and W_E is weight of original effluent sample (g).

Total dissolved solids concentration

Total dissolved solids were determined after drying the samples at 180 °C [158, 159]. Glass fiber filter disks with 42.5 mm diameter and 1.4 μm pore size were first placed on a vacuum filter apparatus (with vacuum in place) and washed with three successive 20 mL portions of distilled water. Vacuum flask and filter apparatus were also washed with distilled water. Disks were dried at 103 – 105 °C in a drying oven for one hour, cooled to room temperature in a desiccator and weighed immediately. Effluent samples were filtered using the above weighed filter disks under a 68 kPa vacuum and washed or filtered three times with distilled water. Total filtrates were transferred to crucibles and evaporating dishes. Filtrates were dried at 180°C in a drying oven, cooled to room temperature in a desiccator and weights measured repeatedly until the change in weight was less than 5% of original. The TDS concentrations were determined using Equation 4.3.

$$C_{TDS} = W_{TDS} \times \rho_E / W_E \quad (4.3)$$

Where, C_{TDS} is TDS concentration in effluent samples (mg/L), and W_{TDS} is weight of final residual after drying the filtrate (mg)

Total suspended solids concentration

Total suspended solids were determined after drying the above disks in the oven at temperature between 103 - 105 °C, cooling to room temperature in a desiccator and measuring the weight [158, 159]. The difference between weights of filter papers before and after filtering the effluent was measured. The TSS concentrations were determined using Equation 4.4.

$$C_{TSS} = W_{TSS} \times \rho_E / W_E \quad (4.4)$$

Where, C_{TSS} is TSS concentration in effluent samples (mg/L), and W_{TSS} is weight of final residual after drying the filter paper (mg).

Fixed and volatile solids concentration

Fixed and volatile solids were determined after igniting residuals from TS and TDS experiments at a temperature of 550 °C [158, 159]. Crucibles and evaporating dishes used for heating the samples were also heated at 550 °C for an hour in a muffle furnace, placed in a desiccator after cooling in air and measured for weight. The cycle was repeated for the samples (inside crucibles) until the weight losses were less than 5% of original weight. TVS concentration values were determined using Equation 4.5.

$$C_{TVS} = W_{TVS} \times \rho_E / W_E \quad (4.5)$$

Where, C_{TVS} is TVS concentration in effluent samples (mg/L), W_{TVS} is weight of final residual after heating to 550 ^{o}C (mg).

4.2.3 Acidity

Acidity of each 5 – 20 mL effluent samples were tested using an Oakton pH 1100 bench top meter equipped with a temperature probe. The meter was calibrated using pH 4, 7 and 10 buffer solutions. Measurements were taken in triplicate for each sample. Acid values of the samples were determined by titrating with a 1 N NaOH solution. A standard 0.05 N sulfuric acid solution was used to standardize the NaOH solution by titration. Acid values were determined using Equation 4.6.

$$AV = V_{NaOH} \times D / W_E \quad (4.6)$$

Where, AV is acid value (NaOH mg/g of sample), V_{NaOH} is volume of NaOH consumed to titrate the effluent samples to within 7 ± 0.1 pH (mL), and D is density of the 1 N NaOH solution (g/l).

4.2.4 Residual Chlorine

Residual chlorine contents were determined for FW1 - C and FW2 - S samples using the Iodometric method [160]. Five to ten grams of samples in triplicate were diluted using 50 mL of distilled water in graduated flasks and 5 mL of acetic acid added to bring the pH level to between three and four. One gram of potassium iodide was added to the samples and shaken vigorously. Titration was carried out in a fume hood. A 0.025 N sodium thiosulphate solution was used to titrate the samples until the yellow colour of the samples disappeared. One milli

litre of starch solution, (prepared by dissolving 5 g of starch in 1 L of boiling distilled water and allowing to settle overnight) was added to the samples and titrated again with the same sodium thiosulphate solution, until the blue colour disappeared. A blank titration was also carried out for a distilled water sample with similar volume using the same procedure. Since a blue colour did not appear after addition of potassium iodide to the distilled water, titration was carried out with a 0.0282 N sodium thiosulphate solution until the blue colour appeared and the 0.025 N solution was used to back-titrate until the blue colour disappeared. The difference between the amounts of sodium thiosulphate added for the blue colour to appear and to disappear was used as the blank titration value. Residual chlorine values were determined using Equation 4.7.

$$RC = [A \pm B] \times N \times 35.45 / V_E \qquad (4.7)$$

Where, RC is residual chlorine in effluent (mg/mg), A is total volume of sodium thiosulphate used for titration of sample (mL), B is amount used for blank titration (mL), and N is normality or gram equivalents of sodium thiosulphate in the solution

Amounts of residual chlorine for FW1 - C, FW2 - S, FW3 - RS and FW3 - FS samples were determined by a slightly different method required as a pre-requisite for the BOD_5 test [160]. Twenty mL samples were first kept in the fume hood for an hour for the residual chlorine to dissipate and, 10 mL of 1 N glacial acetic acid and 10 mL of potassium iodide solution were added [160]. Potassium iodide solution was prepared by dissolving 10 g in 100 mL of distilled water. Effluent samples containing the solutions were titrated using a 0.025 N sodium sulfite solution to the starch-iodide end point. The starch iodide end point was determined by adding 1 mL of the above prepared starch solution and titrating until the blue

colour disappeared. The total amount of sodium sulfite consumed was estimated as the amount required in destroying residual chlorine in BOD_5 test samples.

4.2.5 BOD_5

Rinsed and dried, 300 mL capacity BOD glass bottles with glass stoppers were used for sample incubation. Distilled water used for diluting the samples and solutions were saturated with DO, by aerating with clean compressed air in a partially filled bottle. Ten mL samples were first neutralized to 7 ± 0.1 pH using, standard 1 N NaOH solution. Seeding material was not added since all samples were biological in nature. Test samples were prepared in BOD bottles by diluting 10 mL of the above neutralized effluent samples to 300 mL, using distilled water. Higher residual chlorine amounts were not detected in samples or distilled water as pre determined from the residual chlorine test, therefore sodium sulfite was not added. Phosphate buffer, magnesium sulfate, $CaCl_2$ and ferric chloride solutions were added in 0.3 mL (1 mL/1000 mL of sample) to the samples as required by the standard BOD_5 test [160]. Solutions were prepared as given below:

Phosphate buffer solution: 8.5 g potassium dihydrogen phosphate (KH_2PO_4), 21.75 g dipotassium hydrogen phosphate (K_2HPO_4), 33.4 g disodium hydrogen phosphate heptahydrate ($Na_2HPO_4.7H_2O$), and 1.7 g ammonium chloride (NH_4Cl) dissolved in distilled water and diluted to 1000 mL. The pH was 7.2.

Magnesium sulfate solution: 22.5 g of magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) dissolved in distilled water and diluted to 1000 mL

Calcium chloride solution: 27.5 g of anhydrous calcium chloride ($CaCl_2$) dissolved in distilled water and diluted to 1000 mL

Ferric chloride solution: 0.25 g of ferric chloride hexahydrate ($FeCl_3$. $6H_2O$) dissolved in distilled water and diluted to 1000 mL

Initial DO levels of some samples were lower then 1 mg/L, therefore, new samples were prepared with lower dilution by using 1 mL each from effluent samples and diluting in 300 mL of distilled water, in the BOD bottles. Samples were neutralized and initial DO levels measured after 15 min of preparation using a digital DO meter. Testing was carried out for FW1-C, FW2-S, FW3-R and FW3-F in triplicate. Three blank samples were also prepared similarly. Buffer solutions were added in 0.3 mL each to all samples; bottles tightly corked and kept in a water bath at 20 ± 0.5 °C. Light penetration was prevented by using a dark cover. The DO levels were measured again after 5 days. Equation 4.8 was used for estimating BOD_5 values.

$$BOD_5 = (DO_a - DO_{a5})/ c \quad (4.8)$$

Where, BOD_5 is the five day BOD (mg/L), DO_a is initial DO of samples, DO_{a5} is five day DO level samples, and c is dilution level (In this case it's 300 times for all samples)

4.2.6 Lipid composition

Extraction of lipids from effluent, lipid class composition analysis, derivatization of lipids, and lipid fatty acid composition analysis were carried out at the Ocean Sciences Centre.

Lipid extraction

A triplicate of 5 mg each from FW1 - C, FW2 - S, FW3 - RS and FW3 - FS samples were lipid extracted using standard methods [28]. All containers were cleaned three times using 2

mL of methanol and 2 mL of chloroform. Samples were transferred to round bottomed test tubes and 8 mL of chloroform, 4 mL of methanol, 4 mL of 2:1 chloroform: methanol and 2 mL of chloroform extracted water were added. Solvents and sample and lipid containers were placed in an ice-cold bath during the extraction process. Samples were vortexed for 30 s, sonicated for 10 min in an ice-water bath and centrifuged for 2 min at 5000 rpm. The bottom layer was extracted using double pipettes and transferred to round bottom flasks. Extractions were repeated twice with only 8 mL of chloroform and the pipettes were washed with 1.5 mL of chloroform each in to the round bottom flasks. Solvents (from extracts) were evaporated using a rotary evaporator at a temperature of 40 °C under vacuum until the solutions were almost dry. The residuals were washed with three successive portions of 2 mL chloroform each and transferred to 10 mL calibrated vials. Due to the higher concentration level, the extracted lipid samples were diluted to 10 mL using chloroform. The lipid vials were lightly blown with nitrogen, teflon taped and stored at -20 °C until analysis. A blank sample was also prepared similarly.

Lipid composition analysis

Total lipid content and nine classes of lipids; HC, wax ester/steryl ester (WE/SE), KET, TAG, FFA, ALC, ST, acetone mobile polar lipids and PL were analyzed quantitatively and qualitatively. A nine component standard from Sigma Chemicals (St.Louis, Mo., USA) was used for the calibration and the composition is given in Table 4.2.

Table 4.2: Components in standard used for iatroscan calibration

	Lipid class	Concentration g/l	Abbreviation
Nanodecane	Hydrocarbon	1.1060	HC
Cholesteryl Stearate	Wax ester/steryl ester	0.9708	WE/SE
3-Hexdecanone	Ketone	2.0900	KET
Tripalmitin	Triacylglycerol	2.0388	TAG
Palmic Acid	Free fatty acid	1.0108	FFA
Cetyl Alcohol	Alcohol	0.9900	ALC
Cholesterol	Sterol	1.0580	ST
Monopalmitoyl	Acetone mobile polar lipids	2.0136	AMPL
Phosphatidylcoline Dipalmitoyl	PL	2.1116	PL

The TLC/FID (Mark VI Iatroscan) was used for spotting and analysis of lipids classes. Data from each scan was collected using PeakSimple software (version 3.67, SRI Inc) [161]. Two racks, with each rack containing ten silica coated chromarods were used. Prior to spotting, the racks were blank scanned three times for conditioning and a fourth blank scan was read using the PeakSimple software. Impurities present on the rods were identified and recorded. A 25 µL Hamilton syringe was used for sample spotting on the rods. The syringe was washed with chloroform and the respective sample/standard three times before use. The rods were developed in solvent systems and scanned in three sections. The procedure used is as given below [28].

Scan 1: For HC, WE/SE and KET

- Rods were focused twice in a 60 mL acetone development system until a narrow band of the sample/standard developed at the lower end of the rods. The chamber containing acetone was rinsed with 40 mL of acetone solution before focusing
- Rods were conditioned and dried in a constant humidity chamber over saturated $CaCl_2$ for 5 min

- The first development system was prepared with hexane: anhydrous diethyl ether: formic acid with 98.5: 1: 0.05 ratios. The glass chamber was again rinsed with 40mL of the solution, and 60 mL of the solution was used for focusing the rods.
- Rods were first focused for 25 min, then conditioned in the constant humidity chamber for 5 min and again focused for another 20 min
- After conditioning for another 5 min rods were scanned to the lowest point behind the KET peak using PPS Scan 22 on the Iatroscan.

Scan 2: For TAG, FFA, alcohol (ALC) and ST

- The rods were conditioned for 5 min after the scan
- The second development system was prepared with hexane: diethyl ether: formic acid with a 79: 20: 1 ratio. After rinsing with 40 mL of the solution, 60 mL was used for focusing
- Rods were focused for 40 min, conditioned for 5 min in the constant humidity chamber and scanned to the lowest point behind the Diacylglycerol peak using PPS 11 scan on the Iatroscan

Scan 3: For AMPL and PL

- After conditioning the rods for 5 min they were developed in the previously prepared 100% acetone development system twice, at 15 min intervals
- They were again conditioned for 5 min
- The third development system was prepared with Chloroform: methanol: Chloroform extracted water with a 5: 4: 1 ratio
- Rods were focused twice at 10 min intervals and conditioned for 5 min
- The entire lengths of rods were scanned using the normal scan.

All iatroscannings were carried out at a hydrogen flow between 195 – 200 mL/min and an air flow of 20 mL/min. During calibration, the above procedure was carried out for the standard and two consecutive rods were spotted with 0.5, 1, 1.5, 2 and 3 μL amounts from the standard in both racks. Results were obtained for each rod in terms of peak areas, using the PeakSimple software. Correlation coefficients between the amount spotted and the peak area results were estimated for each rod, while maintaining R-sq values above 0.95 (Correlation above 95%). Developing and scanning of lipid extracted from triplicate of FW1 - C, FW2 - S, FW3 - RS and FW3 - FS samples were also carried out using the above procedure. Samples FW1 - C and FW2 - S were spotted between 0.5 - 2 μL, and FW1-C, and was reanalyzed after 1000 times dilution, due to the high concentration of WE/SE. Lipids from FW3 - RS and FW3 - FS were spotted in amounts varying from 2, 4 and 6 μL. The tenth rod of each rack was spotted with the standard for the qualitative identification of lipid classes. Regression equations obtained during calibration were used for deriving total and lipid class in μg for each chromarod corresponding to the peak areas. These values were used for determining the percentages by weight from original fish waste samples using Equation 4.9.

$$P_{LE} = [W_r \times V_L] / [W_{ww} \times V_s \times d], \quad (4.9)$$

Where, P_{LE} is percentage by weight of total/ classes lipid content in effluent samples (wt. %), W_r is the lipid weight in each chromarod per the area as determined using calibration equations (μg), V_L is total volume of lipids extracted from each fish waste sample used (L), V_s is the volume of sample spotted on each chromarod (μL), and d is the dilution factor

Derivatization of lipids to the fatty acid ester form

Between 200 and 250 µL each from extracted lipid samples were added to lipid cleaned glass vials and evaporated by nitrogen blowing, to near dryness and 0.5 mL of hexane and 1mL of 14% BF_3/ methanol (immediately after taken out from cold storage) were added to the samples. Sulphuric acid was not used due possible impacts on the GC columns [27]. Sample solutions were flushed with nitrogen gas, sealed with teflon liners, vortexed for 30 s, sonicated for 4 min and oven heated for 1.5 hours at 85 °C. After heating, 0.5 mL of chloroform extracted water and 1.5 mL of hexane were added. The upper organic layer (FAME) was removed, transferred to 1.5 mL calibrated vials and concentrated to 1 mL by nitrogen blowing. Derivatized lipid samples were stored at -20 °C to avoid decomposition (due to use of BF_3).

Fatty acid composition analysis

Fatty acids were analyzed on a GC/FID (HP 6890) equipped with an autosampler (7683). The GC column (ZB wax+ from Phenomenex, U.S.A), had a length of 30 m and internal diameter of 0.32 µm. The column temperature began at 65 °C and held for 0.5 min, ramped to 195 °C at a rate of 40 °C /min, held for 15 min then ramped to a final temperature of 220 °C at a rate of 2 °C /min. Final temperature was again held for 0.75 min. The carrier gas used was H_2, flowing at a 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 /min. The detector temperature was kept constant at 260 °C. Peaks were identified using retention times from standards purchased from Supelco. A thirty seven component standard with 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) were used as

standards. Chromatogram peaks were integrated using the Varian Galaxie Chromatography Data System (version 1.9.3.2). Only peaks corresponding to the fatty acid components in the standard were analyzed. Percentage compositions of the individual and classes of fatty acids were determined Equation 4.10.

$$P_{FA} = A_{FA} / \text{Total } A_{TFA} \quad (4.10)$$

Where, P_{FA} is FA by percentage weight of effluent samples (wt. %), A_{FA} is peak area of individual or fatty acid class and A_{TFA} is the total area of identified/unidentified peaks

4.2.7 Effluent from oil recovery process

Total residuals of the waste from the final oil recovering process for samples FW3 - FS and FW3 - RS were collected, mixed and used as the effluent from the oil recovery process. They were analyzed for acidity, TS, TSS, TDS and TVS using the above described methods. Filtrate from the purification process was not incorporated to the effluent.

4.3 Recovery of oil

4.3.1 Test runs using physical separation methods

The process used in the fishmeal industry for recovering oil from fish waste was used as the base [46]. Test runs were conducted using 80 – 83 g of FW1 (30g of FW1 – C and 50g of FW1 – S) samples. Mixing or a second round of grinding, heating, filtering and pressing the solids, and centrifuging was used as the test experimental set up. Heating temperatures of 65, 75 and 90 ^{o}C and heating times of 10, 20, 30, and 45 min, and 1 h were tested. The product after centrifugal separation was filtered through two types of glass microfiber filters (acid treated and non acid treated Whatman type) under a vacuum using a vacuum filtration set up.

4.3.2 Physical separation of oil

A designed experiment was carried out for testing the effects of; initial method of agitation, heating time, heating temperature, final separation centrifugal speed and time, on the quality and quantity of oil product from effluent. The experiment was carried out for FW1 samples using a fractional factorial design with 2^{5-1} or 16 runs [163]. It was carried out in one block since the conditions remained the same and to allow for analysis of results with minimal interferences. The factors and levels chosen for designed experiment are given in Table 4.3. The combination of runs was generated after randomization using Design-Expert software [163].

Table 4.3: Factors and levels for the designed experiment

Factors	Low level – Actual	High level – Actual	Low level – coded	High level – coded
A: Method of agitation	Mixing	Grinding	-1.00	+1.00
B: Centrifuge time	3 min	5 min	-1.00	+1.00
C: Centrifuge speed	1500 rpm	2500 rpm	-1.00	+1.00
D: Heating time	30 min	45 min	-1.00	+1.00
E: Heating temperature	75 °C	90 °C	-1.00	+1.00

The FW1 samples were taken from cold storage and thawed for 30 min in the fume hood. 150 mL amber glass bottles were cleaned by washing with detergent and drying and, again washing with acetone and drying before transferring 20 g of FW1 - C and 65 g of FW1 - S into the bottles, and stored at 3 °C until processing. The experimental procedure is outlined in Figure 4.1.

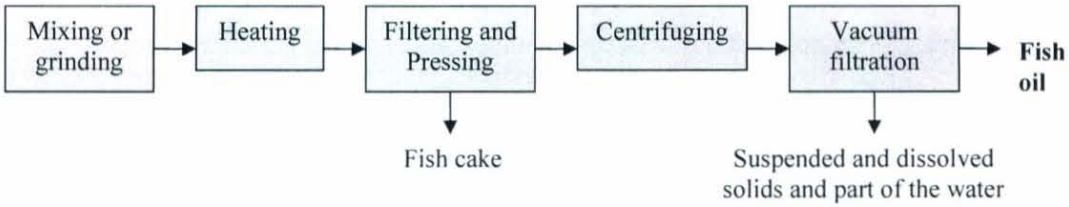


Figure 4.1: Process Flow diagram used for the initial experimental set up

Grinding was carried out in a lab blender at low speed for 3 min and high speed for 1 min. Mixing was carried out on a shaker rack for 30 min before heating. An air oven was used as the heating medium and before heating samples the oven was preheated at the required temperature for one hour. Filtering and pressing were carried out using a higher pore size filter paper (Whatman No.2) and manually pressing the solids to separate the liquid fraction to simulate pressing as in the fishmeal industry. Centrifuging was carried out in an Eppendorf vertical 5810 centrifuge, with a swing bucket rotor of A-4-62. Glass microfiber filters (Whatman, grade GF/C) with 1.2 μm pore sizes and 47 mm diameter were used to separate residual impurities. A fritted glass funnel on filter holder, fixed to a vacuum filtration flask was connected to a vacuum pump for carrying out filtration. Losses of waste/oil in the sample containers, filtration flasks, centrifuge tubes and funnels were also measured. Percentage products recovered were estimated using Equation 4.11. The product here refers to the final output and can have oil as well as other impurities.

$$P_{YR} = W_{fY} / W_{ow} \times 100\ \% \qquad (4.11)$$

Where, P_{YR} is the percentage product or oil recovered (wt. %), W_{fY} is weight of the final product/oil (g), and W_{ow} is the initial waste sample weight (g).

4.3.3 Design optimization using center points

Testing for the percentage product recovered, was also carried out for the average of low and high levels of the centrifuge time (4 min), centrifuge speed (2000 rpm), heating time (37.5 min) and heating temperature (82.5 °C) after grinding or mixing of the waste. Design-Expert software was used for randomizing the run combination and a total of ten runs were carried out (five repetitions) [163]. Vacuum filtration was carried out using an acid treated glass micro fiber filter (Ahlstrom) with 0.7 μm pore size and 47 mm diameter. The experimental run combination is given in Appendix - B.

4.3.4 Fishmeal process simulation

Further simulation of the fishmeal process parameters was carried out for FW2 - S samples. Sample sizes of 100 g were used. A water washing step was added as a final step in addition to the process shown in Figure 4.1, where hot water at 90 °C was added followed by centrifugal separation of the water. Three batches of experiments were carried out for quadruple of samples. Table 4.4 outlines the process parameters. Heating was carried out using a stirred oil bath on a hotplate. Top layer of oil was separated using the double pipette technique after the first and final centrifugal steps.

Table 4.4: Process parameters tested for oil recovery from FW2 - S

	Heating temperature	Time for heating to temperature	Slurry centrifuging speed	Slurry centrifuging time	Oil + hot water centrifuging time	Oil + hot water centrifuging time
Batch 1	90 °C	20 min	1500 rpm	10 min	5000 rpm	5 min
Batch 2	75 °C	15 min	1500 rpm	10 min	5000 rpm	5 min
Batch 3	No heating		1500 rpm	10 min	5000 rpm	5 min

4.3.5 Recovery of oil and purification

The FW3 - RS and FW3 - FS samples were stored at 4 °C for 3 days and thawed in the fume hood for half an hour. Five samples each were used with 80 – 100 g of sample sizes. Samples were transferred to graduated flasks and immersed in an oil bath maintained at 100 - 110 °C. A uniform temperature of 75 °C was allowed by heating to 80 °C in 15 – 20 min. The oil bath stir rate was kept at 200 rpm using a hot plate equipped with stirring. Centrifugation, separation of the oil layer and water washing were carried out at the same conditions as described in section 4.3.4. Recovered oil samples were transferred to amber glass vials, flushed lightly with nitrogen and stored at 1-4 °C storage until further processing (for 3 weeks). Weights of crude oil products were recorded and percentage product recovered estimated using Equation 4.11.

Test runs for removal of impurities in the recovered oil samples were carried out again with an acid treated glass micro fiber filter (Ahlstrom) with 0.7 μm pore size and also using 0.2 μm pore size nylon membrane under vacuum. Oil samples were also filtered using a PTFE membrane (Whatman) with 0.2 μm pore size and 47 mm diameter under a vacuum of 68 kPa, using a vacuum filtration set up. Filtrates were transferred to amber glass vials, flushed with nitrogen and stored at 1-4 °C cooler room. Some samples used 2-3 membranes due to the lower filtration rate. Time taken for obtaining a set amount of filtrate for each sample was measured and recorded along with the number of membranes used and weight of product. The mass flow rates of the samples through the membranes were estimated using Equation 4.12.

$$MF = Y_{fil} / [T \times \pi \times (D_m / 2)^2 \times n_m] \quad (4.12)$$

Where, MF is mass flow rate (kg/m^2. h), Y_{fil} is final filtrate from each sample (kg), T is time taken for filtering (h), D_m is membrane diameter (m), and n_m is number of membranes used.

4.4 Analysis of the oil

4.4.1 Physical properties

Density (Bulk density and specific gravity)

Specific gravity measurements were only conducted where samples volumes were sufficient: FW1, FW2 - S, FW3 - RS and FW3 - FS samples at 25 °C. FW3 - RS and FW3 - FS samples were also measured at 40 °C. A 10 mL volumetric flask was washed with distilled water, dried and measured for weight. A water bath was maintained at 25/40 °C using a hotplate, stir bar and a thermometer. Density measurements were first carried out for distilled water. A volumetric flask was filled with distilled water to the 10 mL level and completely (up to 10 mL level) immersed in the water bath until the sample temperature stabilized at 25/40 °C for 10 min. The flask was removed, wiped and the volume adjusted immediately after to the 10 mL level using a small pipette. Weights were measured. The same procedure was repeated for recovered oil samples and the weights recorded. Specific gravity values were estimated using Equation 4.13.

$$SG = W_{okv} / W_w \quad (4.13)$$

Where, SG is specific gravity, W_{okv} is weight of 10 mL of product or oil sample (g), W is weight of 10 mL of water (g).

Bulk density measurements for the oil were carried out by using methods described in section 4.2.5 for 1 mL and 2 mL samples using Equation 4.14.

$$\rho_o = W_o / V_o \qquad (4.14)$$

where, ρ_o is bulk density of product or oil (g/L), W_o is weight of product or oil (g), V_o is volume of the sample used (L).

Viscosity

A Brookfield DV-III Ultra Programmable Rheometer equipped with a small sample adapter (SSA) and spindle no. SC4-18/13RP was used for measuring dynamic viscosity of the recovered oil samples [164]. The product from FW1 (FW1 – C and FW1 – S mixed samples) from the designed experiments and purified oil from FW3 – RS and FW3 – FS samples were tested for viscosity. The rheometer was calibrated by sending it to CAN-AM instruments Ltd and was auto zeroed at each start up. A temperature probe was attached to the rheometer and viscosities were measured at both 25 °C and 40 °C. A circulating water bath (NESLAB EX series) was connected to the small sample adapter inlet and outlet ports using Nalgene tubing to maintain the required constant temperature. Before taking measurements, the circulating water bath was brought to operation and the set point temperature was kept at the required temperature. Since limitations prevented connecting the cooling lines and regulating the temperature at the set point, the temperature probe was used to maintain the temperature of the sample at ± 0.5 °C of required value. This was achieved by keeping the bath covers open and shutting off the circulating water bath at alternate times.

Oil samples were removed from cold storage and allowed to equilibrate to room temperature prior to testing. The sample chamber and spindle were washed with distilled water, wiped and dried before adding the samples. A sample volume of 6.7 mL was used as per the requirement, when using the SSA with spindle No. SC4-18/13RP [165]. Preparation of the sample setup was carried out by fixing the chamber with oil to the SSA, immersing the spindle slowly in the oil, and subsequently fixing it to the connected rod. Measurements were taken by changing the speed of the spindle from 20 rpm to 240 rpm at intervals of 20, while keeping the motor switched on at all times. Corresponding viscosity values at both 25 °C and 40 °C were recorded in cP at definite time intervals. Measurements were taken at increasing as well as decreasing shear rates and the shear rates also recorded. Kinematic viscosity values of the oil samples were estimated using Equation 4.15.

$$\nu = \mu / SG \tag{4.15}$$

Where, ν is kinematic viscosity of product or oil (cSt), μ is dynamic viscosity of product or oil samples (cP).

4.4.2 Thermal properties

The product recovered from FW1 (FW1 – C and FW1 – S) and purified oils FW3 – FS and FW3 – RS were tested for thermal properties due the representativeness of whole fish waste and salmon gut material. Measurements for melting points, specific heat capacities and detection of decompositions and presence of impurities were carried out using a DSC (TA instruments, Q100 series) equipped with a quench cooling accessory (QCA). Dry ice was used as the cooling medium and measurements were carried out for a temperature range between -55 and 80 °C. Oil samples were placed inside aluminum hermetic pans and sealed

using a sample press equipped with a hermetic die. Tests were carried out in an inert environment by purging the furnace area with 99% purity Nitrogen (obtained from Air Liquide). Gas was connected to both base purge and one of the gas ports, and a standard average gas flow rate of 50 mL/min was used. The gas flow rate was set using the Thermal advantage – Instrument Explorer Software (TA Instruments). The instrument software was used to set the DSC to the new testing conditions and T1 signal. Measurements were carried out under ramp conditions. Samples pans were placed on the cells in the furnace area, and covered with an inner and an outer lid. The QCA with dry ice was placed prior to starting the run sequence and when the temperature of the cell reached -40 °C, a few drops of acetone were added to improve the rate and uniformity of cooling. When the required lower temperature limit was reached the QCA was removed and the furnace covered with the outer cell cover. The run sequence was started at this point. Customized run methods were used for creating required run segments and are described under corresponding sections below. After the final temperature was reached, the cells were cooled to 40 °C using cooling water.

Melting point and decompositions

Melting point calibrations were conducted manually in calibration mode. Baseline temperature and cell constant calibrations were carried out prior to sample testing. Baseline calibration was performed by running empty cells on the entire temperature range used for the tests (-60 to 85°C). The ramping or heating rate was set to 10 °C /min to represent the highest rate at which the samples of interest would be run. The run combination is given below.

a. Sampling interval 1.0 s/point
b. Initial temperature -60 °C

c. Ramp 8 °C /min to 85 °C

d. Isothermal for 1 min

Peak onset and slope values were analyzed in the resulting thermogram and set to the calibration table. Temperature and cell constant calibrations were carried out by placing a standard indium sample of 5-10 mg in an aluminum hermetic pan, and putting this pan and an empty sealed hermetic pan (with similar weight) on the sample and reference cells respectively. The indium sample was run through its melting point by selecting a temperature range of 100 to 200 °C. An average ramping rate of 5 °C /min was used. By setting onset and end point temperature limits of indium melting in the resulting thermogram, the ratio between the actual temperature and the standard indium melting temperature results in a new cell constant value which was entered to the calibration values. Sample runs were conducted between temperatures of -55 °C to 80 °C. All samples were run repeatedly at three different heating rates of; 2, 5 and 10 °C/min and some samples were run 3 times at a particular heating rate. One oil sample recovered from FW1 was heated at 1 °C /min repeatedly to confirm presence of additional peaks. Testing for the purified oil from FW3 - RS and FW3 - FS samples were carried out at temperature range of -60 °C to 150 °C and heating rates of 2, 5 and 8 °C/min. Onset and maximum temperatures for thermogram peaks were analyzed using the Universal analysis 2000 software (TA instruments). Some thermograms were further analyzed using AKTS (Advanced kinetics and technology solutions), an advanced thermal analysis software, to obtain estimates of enthalpy. Peaks having similar onset temperatures at different heating rates were determined as melting peaks. A distilled water sample was also run similarly at 10 °C /min. Enthalpies of melting peaks were obtained by integration. Addtionally, results were verified by testing two samples of the product from

FW1, using an internally cooled DSC1 (Mettler Toledo), at a temperature range of -60 °C to 50 °C and heating rates of 2, 10 and 20 °C/min.

Specific Heat Capacity

Specific heat capacity calibrations were conducted using a standard sapphire (915079.902 Sapphire Specific Heat Material, 0.13 inch OD x 0.020 inch thick, for hermetic pans) obtained from TA instruments. A new cell constant calibration was performed on the experimental mode by setting the previously calibrated cell constant value to 1 on the calibration table. The standard sapphire sample was weighed and sealed in an aluminum hermetic pan. An empty aluminum hermetic pan with similar weight was sealed and used as the reference pan. The two pans were placed in their corresponding places on the cells and the lids put in place. Prior to conducting the calibration, drifts in heat flow were adjusted by conducting a "zero heat flow" segment at 40 °C. Two separate methods were used to conduct calibration runs.

A. Segment sequence of "zero heat flow" method:

a. Sampling interval 1.0 s/point

b. Zero heat flow at 40 °C

B. Segment sequence of method for actual run:

a. Equilibrate at -55 °C

b. Isothermal for 4 min

c. Ramp 10 °C/min to 80 °C

d. Isothermal for 2 min

Both "a." and "b." segments of method B were carried out with the QCA and dry ice in place and removed at the end of segment "b.". The new cell constant value was estimated by the ratio between the actual specific heat capacity of sapphire to the standard value (from a table provided by DSC manuals), where the actual specific heat capacity at 40 °C was calculated as the heat flow normalized to the weight of sapphire and heating rate. The instrument was adjusted for new conditions by entering the new value to the calibration data. A baseline calibration was conducted under these conditions by running method "B" on the experimental mode with empty cells. Testing for the oil samples were carried out using method "B" only. Analysis was conducted using the Specialty Library Software (TA instruments) by simultaneously opening baseline, sapphire and sample thermograms and specifying the region confined to isothermal limits. Graphs and tables presenting specific heat capacity variation with temperature were generated using the same software.

4.4.3 Chemical composition

Lipid composition

Lipid composition analysis was conducted from product from FW1 (mixed samples of FW1 – C and FW1 – S), oil recovered from FW2 – S and purified oil from FW3 – RS and FW3 – FS samples. Lipid class analysis test runs for the recovered product/oil from FW1 were conducted by spotting 0.5, 2, 4, 6, 8 and 10 µL on two consecutive chromarods without any dilution. Additionally, oil recovered from FW2 - S, FW3 - RS and FW3 - FS including FW1 were liquid-liquid extracted before iatroscanning. Using a 1 mL pipette, 1 mL from each oil sample was measured into test tubes. Subsequently, 2mL of chloroform, 1 mL of methanol, 1 mL of 2:1 chloroform:methanol and 0.5 mL of chloroform extracted water were added to the samples. Solvents and extracted lipids were placed in an ice-cold bath during the process of

liquid-liquid extraction. The cycle of vortexing, sonicating, centrifuging and extraction of bottom lipid layer was carried out similar to the methods described in 4.2.6 and repeated twice using only 2 mL of chloroform as the solvent. Second and third washings for oil recovered from FW1 for the centre points of the design were carried out in a separatory funnel by adding chloroform ranging from 10-30 mL for each extraction (depending on the amount needed for a clear biphase separation). Extracted lipids and pipette washings were transferred to 7 mL vials and blown with nitrogen to near dryness. Higher volume extracts were evaporated to dryness using the rotary evaporating system as described in 4.2.5. Residuals were washed three times with three successive portions of 0.5 mL of chloroform each and total washings transferred to 2 mL lipid cleaned and calibrated vials. All three washing were accommodated by blowing with nitrogen. Finally the lipids in the 2 mL vials were evaporated using nitrogen to the 1 mL mark, capped, teflon taped and stored in a -20 °C freezer until analysis. Total lipid and lipid class composition analysis was conducted similar to the procedure described in section 4.2.6. Lipids extracted from FW1 and FW2 - S were spotted on the chromarods in amounts ranging from 0.5 μL - 2 μL. Additionally, lipids from FW1 were reanalyzed in 2 μL spottings after 10,000 times dilution due to the high concentration of WE/SE present. Lipids from FW3 - RS and FW3 - FS were spotted in amounts of 2 μL, 4 μL and 6 μL on the chromarods. Methods given in section 4.4.3 were used for analysis. Derivatization and fatty acid analysis were also conducted for the above 12 samples using methods described in sections 4.4.4 and 4.4.5. Equation 4.16 was used in estimating the percentage by weight of total and classes of lipids in the recovered oil samples.

$$P_{LO} = [W_r \times V_{LO}] / [W_o \times V_{os}] \quad (4.16)$$

Where, P_{LO} is percentage by weight of total/ classes lipid content in recovered oil samples (wt. %), W_r is lipid weight in each chromarod (μg), V_{LO} is total volume of lipids extracted from each recovered oil sample (L), and V_{os} is volume of oil sample spotted on each chromarod (μL).

Total lipid and lipid class recoverability from the waste/effluent samples were estimated for the process described in section 4.4.5 using Equation 4.17.

$$P_{LW} = [P_{LO} \times P_{RY}] / P_L \quad (4.17)$$

Where, P_{LW} is the percentage by weight of lipids or lipid classes recoverable from the waste or effluent samples using the process (wt. %).

Fatty acid composition

Fatty acid composition analysis of the lipids extracted from FW1 (FW1 – C and FW1 – S mixed), FW3 - RS and FW3 - FS was carried out after derivatizing using methods described in section 4.2.6. The products from FW1 were analyzed using a 17 component standard while the standard used for FW3 was the same as used for the waste samples (section 4.2.6).

Sulphur content analysis

Elemental sulphur analysis of the samples was conducted for the purified oil of FW3 – RS and FW3 – FS and was using an Inductively Coupled Plasma - Optimal Emission Spectrometer (Perkin Elmer, Optima 5300 DV ICP-OES machine). About 1 g of the purified oil was diluted to a total weight of 10 g by adding 0.2 g of 100 ppm Mn, and a kerosene

based PremiSolv solvent [166]. Both 10 ppm and 100 ppm standards and calibration blank samples were prepared similarly. A 1 ppm Mn was used as the internal standard and added to the solutions [166]. All samples, standards and controls were vortexed before testing. The instrument tubing (ICP-OES) were purged with argon for 1.5 hours and samples were injected manually. Measurements were set to take three replicates. Sulphur content was determined by analyzing the 181 nm wavelength on the axial emission spectrum.

Moisture content

Three methods to measure moisture content were performed and compared for; the air oven method (AOCS: Ca 2c-25), the modified vacuum oven method (AOCS: Ca 2f-93) and lyophilizing [167]. The three tests were performed for the product recovered from FW1 (FW1 – C and FW1 – S mixed sample) as an initial investigation. The procedure described in section 4.2.2 for total solids testing was repeated for moisture testing using the air oven method, where the moisture was estimated using Equation 4.18. Product sample sizes of 5 – 10 g were used for the AOCS: Ca 2f-93 method (modified method for determination of moisture and volatile matter in fats and oils). Sample containers were prepared by washing, air drying and weighing a 25 mL size vacuum filter flask with a small stir bar inside. Samples were transferred to the containers and a few drops (1-2 mL) of acetone added. Flasks were covered with stoppers and connected to a vacuum pump via a reflux/condenser flask or gas wash bottle to condense and capture some of the evaporated water. A vegetable oil bath with a stir bar on an electric hot plate was used for heating. The stirrer was set to a rate of 4, and the temperature of bath and oil samples was regulated. Samples were gradually heated from 60 °C to 100 °C in 10-15 min, by immersing the sample flask, when the oil bath reached between 60 - 70 °C, and also setting the heating rate to 4. Heating was carried out under

vacuum until complete removal of moisture and the temperature was regulated using the heating rate of the hot plate. Samples were removed from the bath after 30 – 45 min, while keeping the vacuum on. Samples were allowed to equilibrate to room temperature with the stopper on. Stoppers were subsequently removed and outside of the flasks wiped to remove remaining oil from the bath. Weights of the residuals were measured and the percent moisture content in sample was estimated using Equation 4.18. The procedure was repeated for several samples of the recovered oil.

$$P_{VM} = W_{EO} - W_{EF} / W_{EO} \quad (4.18)$$

Where, P_{VM} is volatile and moisture matter of oil or product (wt. %), W_{EO} is weight of original effluent sample (g), and W_{EF} is weight of residual or final sample (g).

Freeze drying or lyophilizing the oil samples were carried out using a 12 L Cascade freeze dry system (Labconco FreeZone Plus, catalog No. 7960040) at the Department of Earth Sciences. Samples were placed in glass vials, weighed and pre-frozen to -10 °C overnight. Subsequently, vials were removed from storage, uncapped, covered with perforated aluminum foils and placed inside freezer dryer bottles. The bottles were placed on the manifold of the freeze dryer and connected to the collector operating at a vacuum of 1 Pa and a temperature of -87 °C for about 3 h.

4.5 Optimization of oil recovery process and Life cycle analysis

The lipid and fatty acid composition of FW1 – C, FW2 – S, FW3 – FS and FW3 – RS were statistically compared using 1 – Way ANOVA and Mann Whitney tests in MINITAB 15 to determine the waste with the highest triacylglycerides (TAG) and monosaturated fatty acids

(MUFA) and the lowest free fatty acids (FFA), phospholipids (PL), wax/steryl esters (WE/SE) and saturated fatty acids (SFA). The results for biological oxygen demand, total solids, total suspended solids, total dissolved solids and moisture of the waste were compared similarly, to determine the effluent samples with the lowest percentage moisture, solids and BOD. The relative standard deviation (standard deviation/ average of results) of the results were used for investigating the heterogeneous nature of samples.

Results obtained from quantitative analysis such as; percentage product recovery and percentage lipid recovery were statistically analyzed to obtain optimum process conditions. The heating time and temperature, centrifuge time and speed and membrane type and pore size that give the highest recovery of the total product, TAG and MUFA and the lowest recovery of FFA, PL and WE/SE were determined by comparing results from the oil/product composition results. Design-Expert (2 – Way ANOVA at 5% significance level) was used for the analysis of product composition results of FW1, where a designed experiment was used. Comparison of results from the product of FW2 – S was using 2 – Way ANOVA in MINITAB and at 5% significance level. This analysis determined the optimum heating rate, heating time and the effect of separating the water in the initial centrifuging stage. The optimal conditions were used for the recovery of oil from FW3 – FS and FW3 – RS.

Lipid and fatty acid composition, and melting points, specific heat capacity, viscosity and density results from FW3 – FS and FW3 – RS were compared using Mann Whitney test in MINITAB to determine the effect of initial freezing on the composition and properties of the oil product. A statistical significance of 5% was used for determining the differences. Again high TAG, MUFA and low FFA, PL, WE/SE, SFA contents; negative melting points and low

specific heat capacity, viscosity and density were preferred in the product. The sulphur content, density and viscosity of the product/oil from FW3 were compared with petroleum fuels (fuel oils, diesel oil) to investigate the applications.

The life cycle reductions in constituents of effluent through recovery of oil were estimated by comparing residual effluent quality data, before and after the optimized physical separation process using 1-way ANOVA and Mann Whitney tests in MINITAB 15, at the 5% significance level. Energy consumption values for the final oil recovery process were estimated using optimized process parameters. Life cycle emission reductions associated with partial or full substitution of the recovered oil in combustors and diesel engines were estimated using published values in NREL, UNISEA fish oil demonstration project and CANMET studies [13, 14, 16]. Reductions in solid/liquid waste and emissions were evaluated for estimating net environmental benefits, and for conducting improvement assessments.

Chapter 5

Results and Analysis

5.1 Characterization of fish processing effluents and residual from oil recovery

The cod whole fish sample (FW1–C) was ash in colour and the salmon gut material samples (FW1 – S, FW2 - S and FW3) were pink. Figures 5.1 and 5.2 present raw salmon waste (FW3 - RS) and frozen salmon waste (FW3 - FS) after grinding and Figure 5.3 presents old salmon waste (FW2 - S) after 3 - 4 months of storage. Grinding FW3 - FS resulted in 3 mm particle size streams of fish waste, while the FW3 - RS was a slurry. The FW2 - S samples (after three to four months of storage) were soft and created negligible back pressure during grinding, which resulted in varying particle sizes. At this stage, it was determined that FW2 - S was rancid, and further analysis was terminated. Results and analysis below for FW2 - S are for waste after one to two months of storage.

Figure 5.1: Ground FW3-RS

Figure 5.2: Ground FW3-FS

Figure 5.3:FW2-S

Waste characterization results below are for cod whole fish (FW1 – C), old salmon gut material (FW2 – S), raw salmon gut material (FW3 – FS) and frozen salmon gut material (FW3 – RS) samples, as well as for the residual waste/effluent from the oil recovery process

of FW3. The residual after recovering oil from FW3 samples are here on referred to as "Re", where Re–FW3 - FS is residual from FW3 - FS and Re – FW3 - RS is residual from FW3 - RS after the recovery of oil.

5.1.1 Bulk density

Bulk density values are given in Table 5.1, and ranged from 1,200 - 2,000 kg/m^3. The density of FW3 significantly reduced after oil recovery with a 19.1% decrease with respect to the median values. Relative standard deviations (RSD) of results even for the same waste/residual ranged between 1 – 33%, where the highest was for FW1. The high deviation indicates to both the heterogeneous nature of the waste and the inaccuracy of using the bulk density method. The measurements are carried out in open test tubes; therefore, entrainment of volatile components of the waste due to high oil content can result in high variability.

Table 5.1: Average bulk density values for the fish waste

Fish waste	Density (kg/m^3)		
	1	2	3
FW 1 - C	1922 ± 24	1721 ± 569	1825 ± 542
FW 2	1407 ± 36	1564 ± 272	1412 ± 31
FW 3- F	1311 ± 50	1429 ± 34	1272 ± 137
FW 3- R	1504 ± 155	1633 ± 241	1381 ± 280
Re – FW3 - FS	1042 ± 250	1054 ± 109	1099 ± 98
Re – FW3 - RS	1194 ± 230	1359 ± 256	1160 ± 206

5.1.2 Solids testing (TDS, TS, TSS, TVS)

Samples after drying are shown in Figure 5.4. Negligible weight changes were observed for the residue samples (Re – FW3 - FS and Re – FW3 – RS) after one to two hours of ignition, whereas the waste samples required more than three hours, indicating a lower organic content in the residue samples.

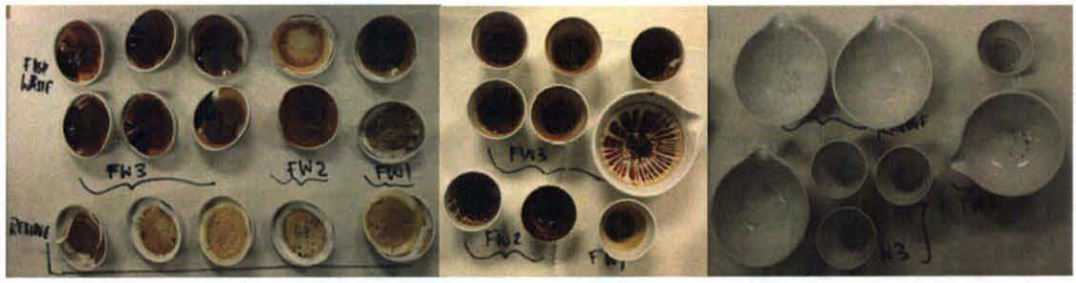


Figure 5.4: Suspended, dissolved solids and ash from FW1 - C, FW2 - S, FW3 and residuals

Solid testing results are summarized in Table 5.2., The total solids (TS) were highest in raw salmon waste (FW3 – RS), among fish waste samples and ranged from 87 to 90 wt. %; and the lowest was in cod whole fish waste (FW1-C) ranging from 33 to 38 wt. %. Lowest overall TS were found in the residual from frozen salmon waste (Re – FW3 – FS), which can be attributed to the separation of the oil layer, thereby reducing solids. The highest moisture content of 74 to 79 wt. % was in the residual. This could be a result of process water added during the oil polishing stage, combined with moisture removed in the oil separation. The ash content of residual and fish waste samples, was less than 1 wt. %, with the residuals higher than waste samples. This indicates less than 1 wt. % of metals in the waste. Higher than 50% RSD were observed in FW1 and FW2 - S for total suspended solids (TSS) and total dissolved solids (TDS), even within the same waste samples, which could be a result of variations during filtering the waste through the membrane, where clogging occurred in some samples.

Table 5.2: Results for average/range of solid waste and moisture in fish waste (wt. %)

Sample (wt. %)	TS	Moisture	TSS	TDS	Ash
FW 1 - C	37 ± 2	63 ± 2	3 - 11	15 - 27	<1
FW 2 - S	57 ± 0.2	42 ± 0.2	3 - 37	10 - 46	<1
FW 3 - FS	83 ± 1	16 ± 1	21 - 23	5 - 9	-
FW 3 - RS	89 ± 1	11 ± 1	5 - 28	6	-
Re - FW3 - FS	22 ± 0.7	78 ± 1	4 - 20	10 - 13	<1
Re - FW3 - RS	24 ± 1	76 ± 1	5 - 14	4 - 15	<1

The values in Table 5.3 illustrate the effect of the oil recovery process on the solid composition of the waste. The total solids in the waste ranged from 667, 561 – 1, 334, 553 mg/L and total suspended and dissolved solids were 130, 095 – 280, 511 mg/L and 75, 389 – 405, 644 mg/L respectively. Total solids were higher than values reported across Canada for fish processing plant effluents (Table 3.1) and can be attributed to the analyzed samples containing mainly solid fish parts resulting in higher solid concentration than the actual plant effluent.

Table 5.3: Results for average solid waste content of fish waste and residual

Sample	TS (mg/L)	TSS (mg/L)	TDS (mg/L)
FW 1 - C	667,561	130,095	374,610
FW 2 - S	817,120	235,640	405,644
FW 3 - FS	1,121,143	280,511	133,479
FW 3 - RS	1,334,553	234,490	75,389
Re – FW3 - FS	233,076	103,260	126,725
Re – FW3 - RS	293,149	105,398	109,072

Total dissolved solids slightly increased and suspended solids decreased after recovering oil from FW3. Since oil generally suspends in the wastewater, recovery of oil results in reducing suspended solids. However, higher molecular weight oil particles could pass through to the filtrate, due to the higher pore size, ending up in the dissolved solids fraction. The decrease of solids was on average 79% for TS and 60% for TSS due to oil recovery from FW3. Total dissolved solids have increased by 44% for raw waste (FW3 – RS) and decreased by 5% for the frozen waste (FW3 – FS) after oil recovery. Removal of dissolved solids in oil recovery can concentrate the amount in the residual (Re – FW3), generally increasing the density; however, due to variations during oil recovery process, part of the oil may possibly dissolve in water.

5.1.3 Acidity

Acidity and pH of the waste and residual are outlined in Table 5.4, acid values were calculated by using a density of NaOH of 1.01 g/mL [168].

Table 5.4: pH and acidity values for fish waste and residual

Fish waste	pH	Acid value NaOH mg/g of sample
FW 1 - C	6.47 ± 0.01	96 ± 12
FW 2 - S	6.09 ± 0.02	182 ± 14
FW 3- FS	6.20 ± 0.04	77 ± 3
FW 3- RS	6.12 ± 0.03	99 ± 15
Re – FW3 - FS	6.32 ± 0.01	37 ± 9
Re – FW3 - RS	6.25 ± 0.03	35 ± 9

Acid values of the waste varied from 45 - 226 NaOH mg/mg of sample and, residuals were 30 - 47 NaOH mg/mg of sample. Both waste and residual pH values were between pH 6 and 7. The highest acidity was observed for old salmon waste (FW2 – S) samples and is likely due to the rancidity. Raw salmon waste (FW3 – RS) was significantly more acidic than the frozen waste. Recovering oil from FW3 lowered the acidity of the wastewater. On average, the acidity of the wastewater decreased by 52% for the frozen samples and 65% for the raw samples after the oil was removed.

5.1.4 Residual Chlorine

The waste itself was yellow, due to the separation of the oil layer, thus, colour changes in the waste were difficult to determine and accuracy of this test was low due to determination of end points through visual changes in colour. Colour changes were not observed for any of the waste samples (FW1 - C, FW2 - S, FW3-RR and FW3-FR) after addition of the potassium

iodide or the starch solutions. Therefore, it was determined that significant residual chlorine amounts were not present in the waste.

5.1.5 BOD_5

The BOD_5, both before and after salinity correction are shown in Table 5.5. Measuring the dissolved oxygen (DO) levels for the fish waste was difficult, as the values did not stabilize, therefore the range at which the values fluctuated, after a constant elapse of time (15 min) was measured. Initial DO levels were less than 1 mg/L for the waste samples after 30 times dilution, resulting in BOD_5 values below the detection limits of the DO meter and, after 300 times dilution, DO levels improved. The final level of dilution is representative of conditions after discharge of waste to river water, which in the literature is 25 – 100% [160].

Table 5.5: BOD_5 values for fish waste samples

Sample name	Range of BOD_5 (mg/L)
FW 1 – C	1230 - 1367
FW 2 - S	309 - 516
FW 3 - FS	23 - 54
FW 3 - RS	5 - 29

The BOD_5 of the waste ranged from 5 – 1367 mg/L. The values reported in literature for BOD_5 of Canadian salmon fish processing plant effluents ranged from 2 to 2600 mg/L (Table 3.1) and measured BOD_5 for the effluent samples are within the range. On average, fresh salmon waste had the lowest BOD_5 across all types of waste, and ranged between 5 – 54 mg/L. The increase in BOD_5 with the time of storage is attributed to microbial degradation and chemical oxidation.

5.1.6 Lipid composition

During the extraction of lipids from the fish waste, several samples showed a thick white layer, which did not dissolve in chloroform and separated to the non-lipid fraction. This layer is possibly a protein rich layer, however this was not tested. The lipid/oil fraction was dark yellow in colour for cold whole fish (FW1 – C) and dark brown for old salmon (FW2 – S) wastes. The lipid concentration after drying of diluted samples was highest in FW3, followed by FW2 - S and FW1-C.

A summary of calibration results in terms of first and second order linear regression; plots, equations and R-squared values for analyzing samples are given in Appendix A. These results were based on final correlations between the peak areas and the actual amount of lipid classes spotted from the standard. The upper boundary limit of the calibration interval for a particular sample was set to twice the largest peak area and the lower boundary as half the smallest peak area obtained during calibrations. Second order regression equations were used for analyzing the lipid content related to large peak areas. Correlations were improved to 0.94 - 0.99, by choosing data points from three calibrations sets, except for FFA in cold whole fish waste (FW1 – C) and old salmon waste (FW2 – S) samples, which was improved to 0.84.

Total lipid and lipid class composition results for fish waste samples are given in Tables 5.6 and 5.7. Iatroscan peaks are shown in Figures in section 5.3 and compared with the recovered oil. Peak areas for wax ester/steryl ester (WE/SE) across all samples and triacylglycerides (TAG) and sterols (ST) of FW3 samples were higher than the upper boundary of the calibration interval. Peaks of WE/SE and ketones (KET) did not separate for analysis of 1 to 5 μL of FW1 - C and FW2 - S, or by diluting samples to 1/1000 and analyzing 1 to 8 μL from

diluted samples. Therefore, the optimum amount of the diluted sample that needs to be spotted for clear and analyzable separation of KET and WE/SE peaks was difficult to determine. However, for diluted FW1 - C samples, the combined shape comprised of several small peaks in the WE/SE region and one peak in the KET region. This indicates to the possible presence of small chain SE (methyl/ethyl/butyl steryl esters), combined with KET that do not separate due to similar polarities. Additional runs were not carried out, and for – cod whole fish and old salmon waste, combined peak areas for wax/steryl esters (WE/SE) and ketones (KET) ranged between 5 – 11 wt. % and 18 – 21 wt.% respectively. Further, using these values total lipids of cod whole fish and old salmon waste ranged between 7 – 14 wt. % and 23 – 28 wt. %. The estimated WE/SE and KET amounts were higher than the calibration interval, and less accurate.

Peak areas for TAG and ST of FW3 were brought within the calibration interval by diluting to 1/10 and analyzing both 1 – 2 μL and 10 – 15 μL of diluted samples. The WE/SE and KET peaks of diluted FW3 samples showed similar patterns to FW1/FW2 - S with peaks in the WE/SE region having areas less than the lower limit of the calibration interval. Errors were minimized by subtracting the blank scan peak areas for WE/SE and KET peaks. Again, this is possibly due to the presence of short chain SE that could also be combined with KET.

Table 5.6: Total lipids and lipid class compositions for FW1-C and FW2 - S (wt. %)

wt. %	FW1 - C	FW2 - S
HC	0.01 ± 0	0.01 ± 0
TAG	0.06 ± 0.01	0.78 ± 0.1
FFA	0.45 ± 0.1	0.10 ± 0.05
ALC	0.20 ± 0.03	1.40 ± 0.1
ST	0.30 ± 0.05	1.28 ± 0.2
AMPL	0.31 ± 0.05	0.85 ± 0.13
PL	1.27 ± 0.14	0.76 ± 0.07

Table 5.7: Total lipid and lipid class compositions for FW3 (wt. %)

wt%	HC	WE/SE + KET	TAG	FFA	ALC	ST	AMPL	PL	Total
FW3 - RS1	0.25%	3.64%	34.96%	1.17%	2.49%	3.70%	1.34%	0.48%	48.02%
FW3 - RS2	0.17%	1.03%	18.33%	1.20%	1.88%	3.79%	1.40%	0.58%	28.38%
FW3 - RS3	0.55%	0.91%	20.27%	3.24%	0.18%	2.82%	1.13%	0.56%	29.65%
FW3 - FS1	0.42%	0.82%	15.20%	1.06%	1.52%	1.36%	0.65%	0.37%	21.40%
FW3 - FS2	0.10%	1.13%	41.05%	1.69%	3.31%	2.37%	0.71%	0.43%	50.80%
FW3 - FS3	0.01%	1.45%	36.02%	1.95%	0.43%	2.18%	0.65%	0.26%	42.94%

Variations of total lipids and lipid classes were observed within samples, for example RSD of TAG for raw (FW3 – RS) and frozen (FW3 – FS) salmon waste samples were 37 and 45% and total lipids were 31 and 40% respectively. The average total lipids by weight were 10.8, 25.1, 35.88 and 38.38 wt. % in cod whole fish (FW1 - C), old salmon (FW2 – S), raw salmon (FW3 – RS) and frozen salmon (FW3 – FS) respectively. This indicated that the non-lipid fraction of cod and old salmon waste samples were much higher than fresh salmon waste. Total lipids were the lowest in cod waste samples. The dominant lipid classes in cod and old salmon waste samples were WE/SE and KET, while TAG were the dominant in fresh salmon waste samples. Average TAG compositions were less than 1 wt. % for both cod and old salmon samples (FW2 - S higher than FW1 - C) and, 24.52 wt. % and 30.76 wt. % for raw and frozen fresh salmon waste. Also, on average, frozen waste had higher TAG than the raw waste, showing frozen storage to be better than cold storage, due to the minimizing of heat related lipid degradation.

A study conducted by Ivar *et al.* (2005) showed the lipid/oil content of pacific cod by-products is 4 wt. %, while cod viscera has 75 wt. % according to Bechtel *et al.* (2006) [10, 12]. The lipid content of 11 wt. %, estimated for the cod whole fish, is lower than literature

values for the viscera. This could be due to the month of harvest, and the part being whole fish, where during the spring months the accumulation of oil is reduced and, the whole fish waste is fish indisposed during the starvation period. The total lipids observed for salmon effluent samples (FW2-S and FW3, between 23 - 50 wt. %) were on average higher than 25 wt. %, the value reported by Sun *et al.* (2006) for Atlantic salmon viscera [111]. This could be due to the variations during sampling and recovery, and samples being the gut material of salmon.

Higher FFA than TAG were found in cod waste, and the opposite for old salmon waste. The cod waste was analyzed after 1 year of storage and old salmon waste after 1 month of storage, therefore, high FFA in cod samples could be attributed to decomposition of TAG. Higher FFA were also present in fresh salmon waste even though freshly analyzed, where in one sample, FFA were greater than 3 wt. %. However, all other fresh salmon samples had less than 2 wt. % FFA. The second highest lipid classes were phospholipids (PL) in cod, alcohols (ALC) in old salmon and sterols (ST) in fresh salmon wastes. The WE/SE and KET compositions in old salmon were higher than the compositions in the cod waste samples, this could be due to storage time where the TAG present in latter could have decomposed.

5.1.7 Fatty Acid composition analysis

Averages of percentage fatty acids by weight of waste samples for FW1 - C, FW2 - S, FW3 - RS and FW3 - FS are summarized in Table 5.8, for three runs. Peaks with retention times beyond an interval defined for a given component in the standard, were placed in the "unknown" category. The majority of the fatty acids were found in the C16 to C18 range and even number carbon chains dominated over odd. The highest fatty acids across all fish waste

samples were C18:1ω9, C16:0 and C18:2 ω6. Additionally, C16:1, C18:0, C20:1, C20:5 ω3 and C24:1 were also present in high percentages. The negligible C20:6ω3 (DHA), across all fish waste types differed from literature however, C20:5ω3 (EPA) amounts in salmon fish waste was in agreement with literature values [10, 35, 129].

Table 5.8: Fatty acid composition of fish waste samples (wt. %) from the GC analysis

	FW1 - C	FW2 - S	FW3 - RS	FW3 - FS
C14:0	2.19 ± 0.16	4.42 ± 0.20	2.57 ± 0.14	2.53 ± 0.05
C14:1	0.07 ± 0.01	0.06 ± 0.00	0.09 ± 0.01	0.09 ± 0.01
C15:0	0.17 ± 0.01	0.31 ± 0.01	0.18 ± 0.00	0.18 ± 0.00
C15:1	0.03 ± 0.01	0.06 ± 0.01	0.02 ± 0.01	0.03 ± 0.00
C16:0	14.52 ± 0.14	16.52 ± 0.42	16.59 ± 0.25	15.50 ± 0.16
C16:1	6.14 ± 0.29	7.08 ± 0.25	8.28 ± 0.32	7.79 ± 0.15
C17:0	0.17 ± 0.01	0.81± 0.04	0.33 ± 0.05	0.37 ± 0.02
C17:1	0.26 ± 0.07	0.82 ± 0.03	0.24 ± 0.01	0.15 ± 0.00
C18:0	4.70 ± 0.14	4.42 ± 0.23	4.43 ± 0.23	4.01 ± 0.08
C18:1 ω9	31.16 ± 2.00	23.32 ± 1.41	32.87 ± 0.97	31.11 ± 0.59
C18:2 ω6	10.36 ± 0.19	8.38 ± 0.14	12.85 ± 0.20	12.78 ± 0.19
C18:3 ω6	0.04 ± 0.02	0.35 ± 0.01	0.15 ± 0.01	0.16 ± 0.02
C18:3 ω3	0.74 ± 0.06	1.07 ± 0.03	0.99 ± 0.05	1.04 ± 0.05
C20:0	0.02 ± 0.02	0.15 ± 0.02	0.07 ± 0.01	0.08 ± 0.00
C20:1	5.33 ± 0.47	1.04 ± 0.75	2.13 ± 0.14	1.75 ± 0.09
C20:2	1.15 ± 0.03	0.46 ± 0.02	0.59 ± 0.03	0.57 ± 0.00
C20:4 ω6	0.40 ± 0.03	0.88 ± 0.06	0.83 ± 0.02	0.94 ± 0.03
C20:3 ω3	0.12 ± 0.00	0.09 ± 0.01	0.05 ± 0.02	0.06 ± 0.00
C20:3 ω6	0.67 ± 0.04	0.28 ± 0.02	0.00 ± 0.00	0.51 ± 0.02
C20:5 ω3	0.88	8.54 ± 0.09	3.04 ± 0.07	3.46 ± 0.13
C21:0	0.10 ± 0.02	0.01	0.49 ± 0.02	0.00 ± 0.00
C22:0	0.16 ± 0.03	0.11	0.09 ± 0.07	0.00 ± 0.00
C22:1 ω9	2.63 ± 1.93	0.40	0.78 ± 0.08	0.16 ± 0.01
C22:6 ω3	0.00 ± 0.00	0.04	0.00 ± 0.00	0.00 ± 0.00
C22:2	0.11 ± 0.01	0.03	0.00 ± 0.00	0.02 ± 0.01
C23:0	0.02	0.01	0.00 ± 0.00	0.00 ± 0.00
C24:0	0.03 ± 0.00	0.04 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
C24:1	1.53 ± 0.11	6.14 ± 0.25	4.52 ± 0.04	5.14 ± 0.26
Unidentified	16.49 ± 15.6	17.48 ± 3.19	7.86 ± 0.61	12.37 ± 1.36
SFA	21.72 ± 0.62	26.65 ± 0.59	24.72 ± 0.26	22.61 ± 0.29
MUFA	43.43 ± 7.84	38.79 ± 2.53	48.94 ± 0.81	45.74 ± 1.44
PUFA	13.15 ± 0.34	17.09 ± 5.11	18.48 ± 0.32	19.28 ± 0.08

The highest class of fatty acid was monosaturated fatty acids (MUFA), which ranged between 35 wt. % and 49 wt. %. The saturated fatty acids (SFA) ranged between 21 and 27 wt. % and were higher than polyunsaturated fatty acids (PUFA) but, lower than MUFA. Variations in fatty acid classes were low (standard deviations were less than 1) in fresh salmon waste compared with both cod and old salmon wastes. Highest MUFA was in raw salmon (FW3 – RS) and lowest SFA was in cod waste, across all samples. The overall fatty acid amount in cod and old salmon were higher than for fresh salmon waste. The MUFA in fresh salmon ranged from 45 - 49 wt. % and higher than for both cod and old salmon wastes. Saturated fatty acids and carbon chains with more than 20 carbon atoms were higher for old salmon than both cod and fresh salmon wastes. These indicate higher levels of decomposition in old salmon waste. Overall, unsaturated fatty acid amounts (55 wt. % to 67 wt. %) were higher than saturated ones (7 wt. % to 18 wt. %) in the waste. The composition of salmon waste, (MUFA higher than SFA and PUFA) differs from composition reported by Sun *et al.* (2006) for farmed Atlantic salmon viscera, where equal percentages of SFA, MUFA and PUFA were found [111]. These differences are likely due to old and fresh salmon wastes being the gut material of farmed Atlantic salmon.

5.2 Recovery of oil: Percentage recovered oil and other observations

5.2.1 Test run results for recovering oil from a mix of cod and salmon waste (FW1)

Heating the waste to 65 °C did not show any apparent separation of the oil fraction from a mix of salmon gut material and cod whole fish waste (FW1 – C and FW1 – S). The temperature of 75 °C was the lowest temperature at which separation of the two fractions was detected (bottom layer of an oil-water emulsion and a top layer of solids). Heating to a

temperature between 75 °C and 90 °C for 10 - 20 min did not show much liquid separation and 30 min was the lowest time in which separation was seen in the mixed sample.

Centrifuging of one year old cod whole fish waste (FW1 – C) showed four layers of separation; a bottom layer of coarse solids, a second layer of an oil-water emulsion, a third layer consisting of a fine solids slurry, and a rather thin top layer of viscous oil. The top layer was only detected at centrifuge times greater than 15 min at centrifugal forces greater than 2000 x g. Figure 5.5 represents the separation after centrifuging at 2500 x g for 30 min. Centrifuging at 3000 x g did not show a significant increase in top layer.

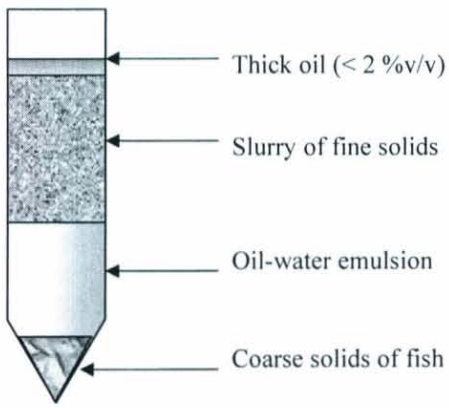


Figure 5.5: Separation of FW1 - C after centrifuging

The thick oil layer was not observed for the fresh cod waste and was attributed to denatured oil separated from the waste. Separation of the water-oil emulsion layer followed by centrifuging did not show separation of an oil layer.

5.2.2 Effect of processing on oil recovery, using a designed experiment

Run time for the oil recovery process was four hours per sample for the mixed cod and salmon waste samples (FW1 – C and FW1 - S). The percentage recoveries for the product (water-oil emulsion layer) varied between a minimum of 3.28 wt. % of waste and a maximum of 13.35 wt. % with an average 7.26 wt. %. Results were statistically analyzed using Design-Expert software, as shown in Appendix B, to identify the effect of different factors and levels on the oil recovered [163]. A Pareto chart and half normal plots available on the software were used to confirm statistical significance of these results. The list of effects with the highest contributions to the percentage product recovery were agitation/centrifuge speed, agitation/temperature, heating time/temperature, heating time, agitation/ heating time, centrifuge time and agitation.

An overview of the results as analyzed in Appendix B using interaction plots follows. Grinding followed by heating for 45 min increased the product, while heating for 30 min slightly decreased the product. The sample should be agitated or mixed, when using the higher heating temperature, and ground when using the lower heating temperature to maximize the percentage product recovery. This could be because at lower temperatures agitation could have a more dramatic effect on blood cell eruption and release of oil compared to higher temperatures. Heating for a higher heating time of 45 min at 75 °C or heating for 30 min at 90 °C increased the product, with the latter combination giving the highest product. Numerical optimization and model equations available on Design-Expert were used to obtain the combination of factors and levels which gave the maximum percentage product. Grinding, followed by centrifuging for 5 min at a speed of 1500 rpm and heating for 45 min at 75 °C gave the maximum percentage product of 13.18 wt. %.

Additionally, product losses during the recovery process were estimated as given in Table 5.9. The confidence intervals at 95% significance level were estimated using Minitab 15 [169].

Table 5.9: Losses/ mass reductions of the waste during the oil recovery process

Equipment	No of data points	Median loss in grams	Lower Confidence interval	Higher confidence interval
Heating flask	16	2.898	2.58	3.27
Grinder	8	12.78	11.39	14.03
Filtration flask	16	0.205	0.16	0.33
Filtration funnel	11	0.243	0.11	0.64
Vacuum flask	8	0.153	0.1	0.205
Centrifuge tube	14	0.215	0.165	0.3

The highest losses occurred during grinding while centrifuge tube losses were due to removal of residual solids. Experimental errors are associated with the filter pressing step and may have resulted in variations in percentage product recovery. Oven temperature stabilization was another challenge as opening the oven when samples were placed resulted in slight drop in the temperature. This was partially mitigated by ensuring the oven was allowed to sit for one hour at the specified temperature prior to introducing the sample.

5.2.3 Design optimization using centre points

The effect of changing filters on the percentage product recovery from the cod and salmon mixed samples (FW1 – C and FW1 – S) was significant (Appendix B), thus effect of recovering oil at averages of above factors of; grinding/mixing, heating for 37.5 min at 82.5 ^{o}C, centrifuging for 4 min at 2000 rpm, was not possible to determine. Percentage product from filtration using the 0.7 μm acid treated membrane was significantly higher than when

using the 1.2 μm non-acid treated membrane. This can be attributed to the acid treated membrane reducing retention of moisture present in oil.

5.2.4 Fish meal process simulation using old salmon waste (FW2 – S)

Separation of old salmon waste (FW2 - S), after the first centrifugation, was similar to the cod waste (Figure 5.9); however the volume of the oil layer was much larger than for the cod waste, and only this layer was separated as the product. Two parameters were tested during the recovery process; 1) the effect of centrifuging/heating and 2) the effect of heating temperature on the recovered oil. Instead of percentage recovered oil, total lipid and lipid class composition values were used for analyzing the effect of these process parameters, and these are discussed under section 5.5.1.

5.2.5 Recovery of oil and purification using fresh salmon waste (FW3 – FS, FW3 - RS)

Separation of the oil layer after the first centrifuging step is presented in Figure 5.6. An orange thick layer of oil makes up the top layer, then a solids layer, and an aqueous layer. Visual observation of the recovered oil after filtering through the 0.2 μm nylon membrane showed fewer impurities in the filtrate. Filtering through the 0.7 μm acid treated glass microfiber membrane resulted in a white residual on the filter, likely proteins. The hydrophilic 0.2 μm membrane showed retention of a small amount of the oil while the 0.7 μm acid treated hydrophobic membrane did not show any liquid retention. Lower pore size (0.2 μm) likely retains some of the heavier oil fractions such as waxes, but not moisture, and acid treated hydrophobic membranes as discussed earlier (section 5.2.3) may result in reducing moisture retention. A 0.2 μm (non-acid treated) hydrophobic membrane can retain both water and heavier oil fractions.

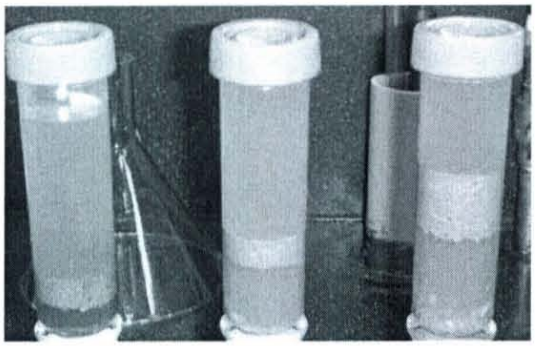


Figure 5.6: Separation of the oil layer from fish waste in initial centrifuging

Percentage recovered oil after purification and mass fluxes for the 0.2 μm hydrophobic PTFE membrane are given in Table 5.10. Applying a 68 kPa vacuum resulted in less than 1 $kg/m^2.h$ mass fluxes through the membrane for some samples, which could be due to the lower filter area (47.5 mm diameter) of the membrane. Fresh salmon samples; FS - 3 (frozen), RS - 3 (raw) and RS - 2 had the highest mass fluxes. Average percentage recovered oil and mass fluxes were; 16.54 ± 5.7 wt. % and 1.27 ± 1.2 kg/m^2 h for the oil recovered from the frozen salmon waste (FW3 – FS) and, 19.83 ± 6.8 wt. % and 1.20 ± 0.69 $kg/m^2.h$ for the oil recovered from the raw waste (FW3 – RS). Percentage recovered oil on average were not significantly different between recovered oil from raw and frozen wastes.

Table 5.10: Percentage recovered oil from fish waste and mass fluxes of membranes

Sample	Percentage recovered oil (wt. % of fish waste)	Mass flux (kg/m^2. h)
FS - 1	24	0.79
FS - 2	17	0.84
FS - 3	14	3.42
FS - 4	8	0.57
FS - 5	19	0.72
RS - 1	13	0.39
RS - 2	19	1.28
RS - 3	31	2.26
RS - 4	20	0.83
RS - 5		1.25

5.3 Analysis of the oil/ recovered oil: physical properties

Product from the cod and salmon whole fish mixed samples (FW1), filtered using 1.2 µm membrane is here onwards referred to as block 1 (no. 1 to 16) and, product filtered using 0.7 µm acid treated membrane (center points of the design) refers to block 2 samples (no. 17 to 25). The products from old salmon waste (FW2-S) are referred to as EX1 - 1 to EX1 - 4, EX 2 - 1 to EX2 - 4 and EX3 - 1 to EX3 - 4. Samples FS - 1 to FS - 5 refers to recovered oil from frozen salmon waste (FW3 – FS) and samples RS - 1 to RS - 5 refer to recovered oil from raw salmon waste (FW3 – RS).

5.3.1 Visual observations

Cod and salmon whole fish waste mixed samples (FW1)

The recovered product was dark light yellow in colour or dark brown as shown in Figure 5.7. Visual observation for colour and presence of sediments are given in Table 5.11, along with heating temperatures during experiments. Relationships were observed between heating temperature and colour, and heating temperature and presence of sediments, as indicated in Table 5.11.

Figure 5.7: Product from FW1 obtained from the designed experiment

Statistical analysis showed that colour differences were correlated to heating temperature. Sediments were, on average, observed in samples heated at the higher temperature of 90 °C than samples heated to 75 °C.

Table 5.11: Visual observation results for colour and presence of sediments

Sample	Heating temp	Apparent colour	Suspended solids
1	90	Dark	none visually
2	75	Light	some turbidity
3	90	Light	present visually
4	75	Dark	No
5	90	Light	present visually
6	90	Light	some turbidity
7	75	Dark	No
8	75	Light	No
9	90	Light	present visually
10	75	Dark	No
11	90	Light	some turbidity
12	75	Dark	No
13	75	Dark	No
14	90	Dark	No
15	75	Light	-
16	90	Light	present visually

5.3.2 Density results (bulk density and specific gravity)

Cod and salmon whole fish mixed samples (FW1)

Density values at 25 °C for block 2 samples are given in Table 5.12. Densities were not measured for block 1 samples due to the insufficient volume in recovered product. Densities of block 2 product ranged between 1050 and 1058 kg/m^3 and were similar to that of water, indicating high water content. Standard specific gravity flasks were not used for measurements; therefore, the accuracy of the densities is low. The density of the product from FW1 is not comparable to literature values due to high moisture content.

Table 5.12: Density results for the block 2 product at 25 °C

Sample	Density (kg/m^3)
19	1055
20	1058
21	1054
22	1055
23	1055
24	1054
25	1051

Purified oil recovered from raw and frozen salmon wastes (FW3 – FS, FW3 – RS)

Specific gravity and density values for the purified oil at 25 °C and 40 °C are given in Table 5.13 for three measurements. Average specific gravities of the raw and frozen oil samples were 0.918 and 0.919 at 25 °C and, 0.914 for both at 40 °C. On average, both purified oils had lower density and specific gravity than water, and RSD values for both temperatures were less than 0.5%. Densities were not significantly different for the purified oil, from raw and frozen waste, at both temperatures. The density of purified salmon oil was similar to bulk density values reported by Sathivel *et al.* (2008) for oil recovered from salmon heads [8].

Table 5.13: Specific gravity and density values for the recovered oil at 25 °C and 40 °C

Sample	25 °C		40 °C	
	Specific gravity	Density (kg/m^3)	Specific gravity	Density (kg/m^3)
R	0.918 ± 0.0	914 ± 1.2	0.913 ± 0.0	907 ± 1.5
F	0.919 ± 0.0	915 ± 2.3	0.913 ± 0.0	907 ± 3.2

5.3.3 Viscosity

Cod and salmon whole fish mixed waste (FW1)

Results for dynamic viscosity of the product at 25 °C are given in Table C-1 (Appendix C) values at 40 °C are given is given in Table C-2 (Appendix C). Figures 5.8 and 5.9 present the changes in viscosity with shear rate for block 1 and 2. Viscosity was inversely correlated to

the shear rate or motor speed, and viscosity for a particular shear rate was consistent, when shear rates are increased from 26.4 to 317 s^{-1} or, decreased from 317 to 26.4 s^{-1}. Viscosity values for the product from both block 1 and 2, ranged between 2 and 5 cP at 25 °C, and close to that of water. Samples no. 2, 6 and 11 followed a similar pattern in decrease of viscosity at 25 °C, and samples; 1, 8, 9 and 15 showed a comparatively higher decrease.

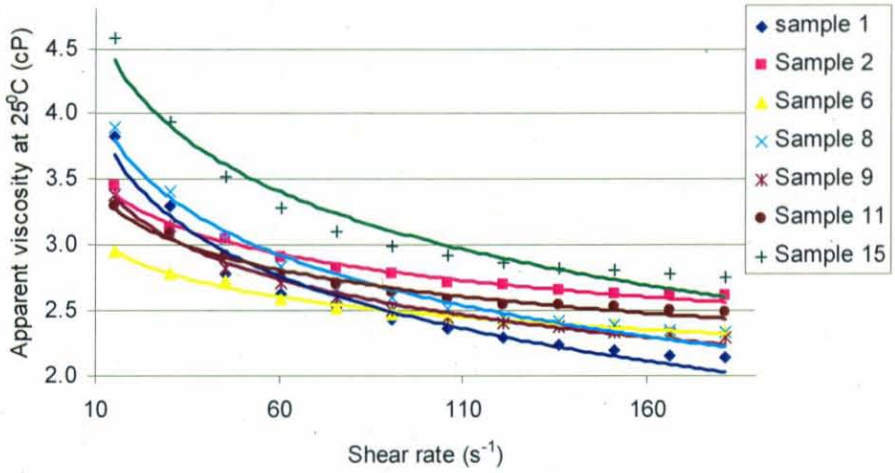


Figure 5.8: Change in apparent viscosity with shear rate at 25 °C for block 1 samples

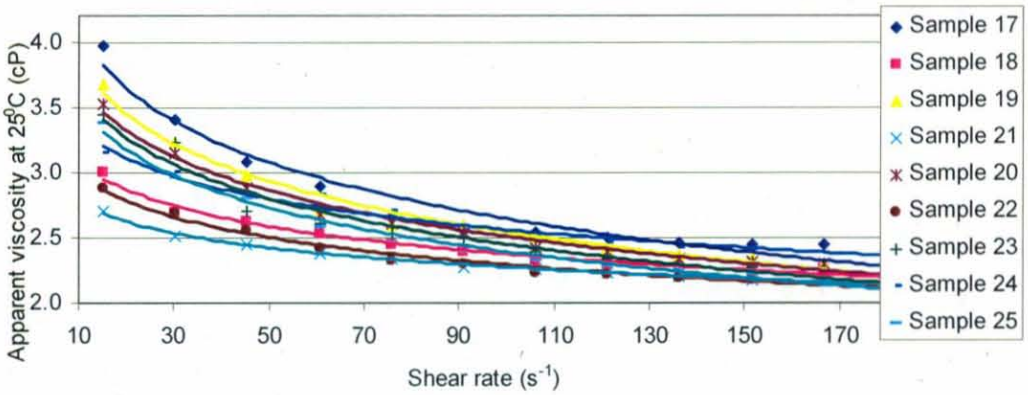


Figure 5.9: Change in apparent viscosity with shear rate at 25 °C for block 2 samples

The percentage decrease in viscosity of block 1 samples was lower than block 2, when temperature is increased from 25 °C to 40 °C. Sample no.2 from block 1 was tested at 40 °C. Viscosity of block 1 and 2 products ranged from 1.5 - 2.5 cP at 40 °C, and the decrease when the temperature is increased from 25 °C to 40 °C, was lower for block 1 products than block 2. The average decrease of viscosity with increase temperature was 29% - 31% for block 1 and 33% - 41% and for block 2 samples.

The patterns observed for apparent dynamic viscosity at 25 °C, for the product from FW1 can be called a "pseudoplastic fluid" [157]. Paints, emulsions and dispersions are typical fluids in the same category; therefore the product is likely an emulsion. The spindle type and the motor speed could affect viscosity, and the initial resistance decreases as the shear is increased, reducing the viscosity. The flow behaviour of the product was further analyzed using flow behaviour indexes. Power and Newton's laws are given in Equations 5.1 and 5.2 and were used for deriving Equations 5.3 and 5.4 [36]. Using equation 5.4, the flow behaviour index values were derived as given in Table 5.14.

$$\sigma = K\gamma^{f} \qquad (5.1)$$

Where, σ is shear stress (Pa), γ is shear rate (s^{-1}), K is consistency index (Pa s^{n}), and f is flow behaviour index.

The following equation relates dynamic viscosity, μ (Pa s) to the shear stress and shear rate.

$$\eta = \sigma / \gamma \qquad (5.2)$$

Where, μ is viscosity (Pa s)

Therefore,

$$\mu = K\gamma f^{-1} \quad (5.3)$$

$$\text{Log } \mu = (f - 1) \log \gamma + \log K \quad (5.4)$$

The flow behaviour index values were less than one and ranged from 0.75 to 0.9 across all product samples confirming the non-Newtonian "pseudoplastic" behaviour, where viscosity decreases with an increase in the shear rate [157]. Flow behaviour index values were in accordance values reported by Sathivel *et al.* (2008) for unrefined pollock oil (0.8 to 0.9) [8].

Table 5.14: Flow behaviour index values for the product from FW1

Sample	Equation	Flow behaviour index
1	y = -0.2357x - 2.1477	0.76
2	y = -0.1129x - 2.3339	0.89
6	y = -0.0981x - 2.4114	0.90
8	y = -0.2144x - 2.1595	0.79
9	y = -0.165x - 2.2726	0.83
11	y = -0.1195x - 2.3404	0.88
15	y = -0.2086x - 2.1034	0.79
17	y = -0.2054x - 2.17	0.79
18	y = -0.1198x - 2.3861	0.88
19	y = -0.1966x - 2.2049	0.80
20	y = -0.1812x - 2.2407	0.82
21	y = -0.0909x - 2.4625	0.91
22	y = -0.1238x - 2.3936	0.88
23	y = -0.1842x - 2.2452	0.82
24	y = -0.125x - 2.3431	0.88
25	y = -0.1832x - 2.2576	0.82

Oil recovered from fresh salmon waste (FW3 – FS, FW3 – RS)

Dynamic viscosity results for the purified oil from FW3 at 25 °C and 40 °C are given in Tables 5.15 and 5.16. Testing at 25 °C was done to a motor speed of 40 rpm as torque

exceeded 100% at shear rates higher than 52.8 s^{-1}, making measurements above 52.8 s^{-1} using the existing set up not possible.

Table 5.15: Viscosity values for the purified oil at 25 °C

	Shear rate - 26.4 (s^{-1})		Shear rate - 52.8 (s^{-1})	
	Motor speed - 20 rpm		Motor speed - 40 rpm	
	Viscosity (cP)	Torque (%)	Viscosity (cP)	Torque (%)
RS	53.05 ± 2.69	35.42 ± 2.51	51.95 ± 1.82	70.68 ± 5.03
FS	51.05 ± 1.29	33.88 ± 0.49	50.72 ± 0.69	67.77 ± 1.19

Viscosity was measured at 40 °C, to a motor speed of 80 rpm, except for three raw waste samples (RS - 2, RS - 3 and RS – 4). Viscosities of RS - 3 and RS - 4 samples were measured up to 100 rpm motor speed. A change of spindle and/or the small sample adapter could have provided a means of measurements at higher motor speeds. Variations in measurements in terms of RSD were less than 5% at both temperatures, except for the torque of purified oil from raw waste. Average viscosities were 52.5 cP for raw samples and 50.9 cP for frozen samples, and values were not significantly different between raw and frozen samples at 25 °C. Viscosities were measured for both purified and the crude oil samples. Crude oil viscosities over the entire shear rates tested were compared for statistical differences with purified samples using Mann Whitney test [169]. Viscosities of the purified oil from raw samples were lower than that of crude oil at 5% significance level. Viscosities were less than that of crude oil for the purified oil from frozen samples, at the 10% significance level. The viscosities of the partially purified oil from raw and frozen waste at 40 °C were 28.2 cP and 29.4 cP respectively. Significant differences in viscosities between raw and frozen samples were not observed over the entire shear rates tested. The viscosity decreased by 44.38%, when increasing temperature from 25 °C to 40 °C.

Table 5.16: Viscosity values for the crude and purified oil at 40 °C

s^{-1}	Shear rate - 26.4		Shear rate - 52.8		Shear rate - 79.2		Shear rate - 106		Shear rate – 132	
rpm	Motor speed - 20		Motor speed - 40		Motor speed - 60		Motor speed - 80		Motor speed - 100	
Sample	μ (cP)	T (%)	μ (cP)	T (%)	μ (cP)	T (%)	μ (cP)	T (%)	μ (cP)	T (%)
RS - purified	29.33 ± 0.50	19.63 ± 0.08	29.38 ± 0.93	37.83 ± 1.32	28.22 ± 1.33	56.82 ± 3.45	27.25 ± 0.07	72.7 ± 0.21	21.17 ± 0.11	90.55 ± 0.21
RS - crude	29.95	19.65	29.30	39.70	29.75	59.25	29.95	80.30	-	-
FS – purified	29.40 ± 0.61	19.47 ± 0.45	28.88 ± 0.46	38.75 ± 0.43	29.23 ± 0.50	58.70 ± 0.7	30.03 ± 0.26	80.18 ± 1.48	-	-
FS - crude	30.05	19.90	29.20	39.35	29.95	59.10	30.15	81.60	-	-

μ – Viscosity

T - Torque

5.2 Analysis of recovered oil: thermal properties

5.4.1 Melting and decompositions

Product from cod and whole salmon mixed waste (FW1- C and FW1 – S)

The DSC thermograms for several samples were obtained using the TA instruments Universal Analysis software for a temperature range between -50 °C and 80 °C and are given in Figure 5.10. A large peak consistent with heating rates was seen for all samples near 0 °C. However, the onset temperature of the peak was not similar to distilled water and could indicate an oil-water emulsion present in the product. Samples 8, 13, 14, 20, 22 and 24 show additional peaks appearing at the lowest heating rate of 2 °C /min, with onset temperature between -30 °C and -40 °C. The additional peaks did not appear consistently at different heating rates. In addition, samples 1, 2, 3, 7, 13 and 25 showed peaks at positive temperatures inconsistent with heating rates. These can be attributed to decomposition, impurities or instrumental errors at higher resolutions. Overall, one melting was seen with an onset temperature between -10 °C and -15 °C.

Since the conventional DSC was equipped with a manual cooling accessory, results were associated with error and augmented when only the T1 signal was used with QCA. Therefore, results were confirmed for samples 2 and 17 using the internally cooled DSC1 and the thermograms are given in Figure 5.11.

Only one peak was observed for both samples within the entire temperature range, whereas sample 2 showed another peak using the conventional DSC and is likely due to an instrumental error. The onset temperature of the peak for samples 2 and 17 using the conventional DSC was similar to results from internally cooled DSC1. An overall summary of the results are given in Table 5.17. Enthalpy values were less accurate as baseline optimization was not carried out using AKTS software and provided only a basic overview of the size of the melting and/or decompositions. Overall, all samples had a confirmed melting between -10 °C and -15 °C. Compared to the literature values for catfish and salmon crude oils (Chapter 3), the upper value of the melting range of the product is lower and, the proximately of one peak to 0 °C confirmed the presence of oil in an emulsion with water.

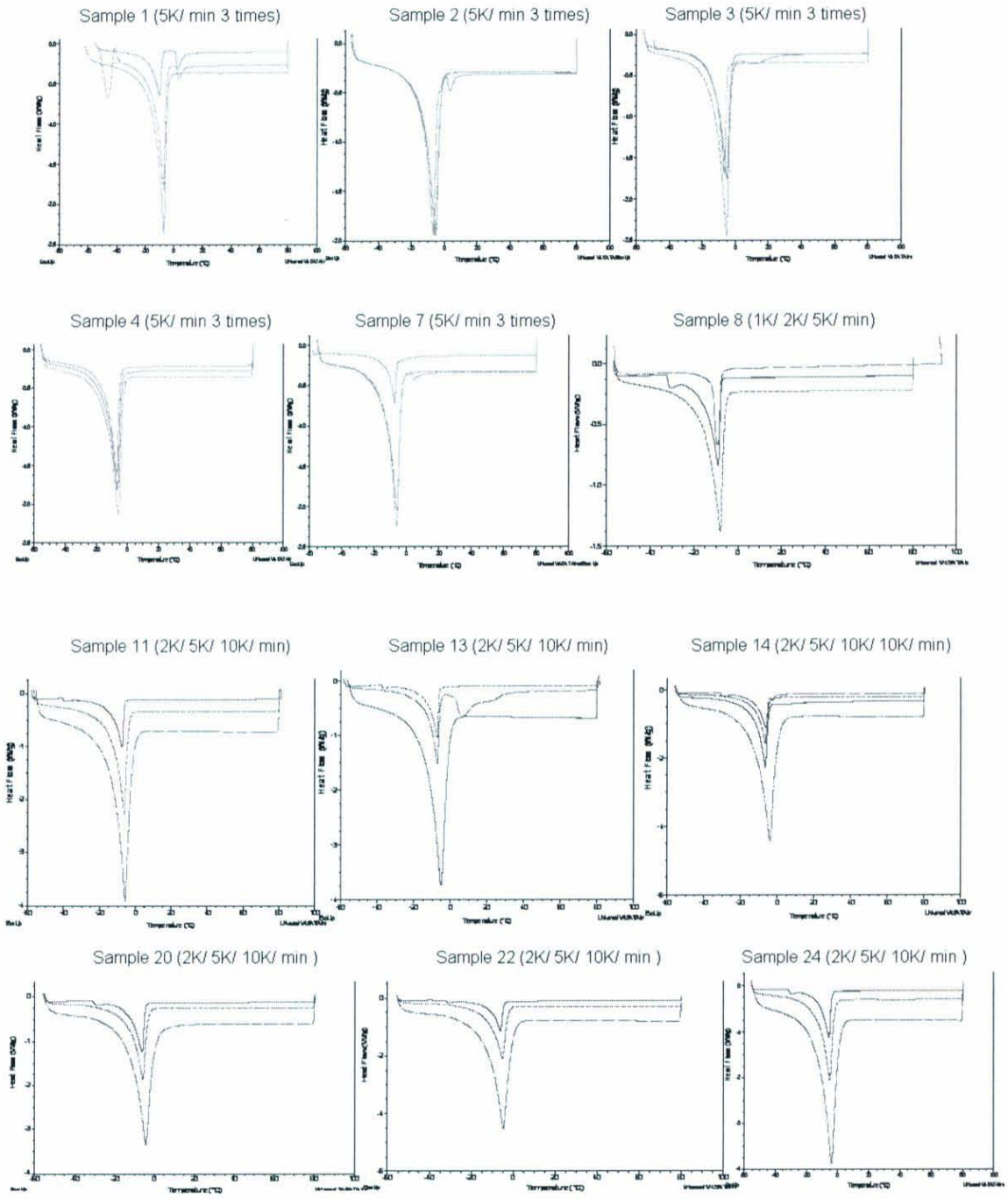


Figure 5.10: DSC thermograms for samples 1, 2, 3, 4, 7, 8, 11, 13, 14, 20, 22 and 24

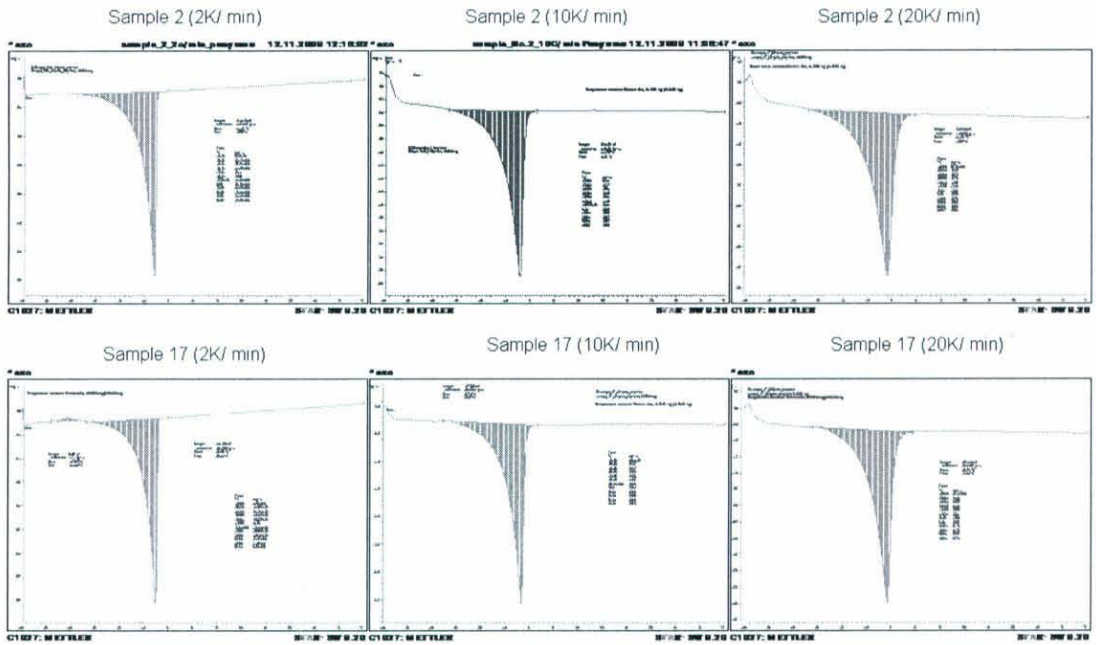


Figure 5.11: DSC thermograms for samples 2 and 17 using internally cooled DSC1

Table 5.17: Melting points and enthalpies of product from FW1 using the conventional DSC

Block description	Sample	Heating rates	Melting points °C		Enthalpy of main peak kJ/kg
			Peak onset	Maximum	
Block 1	1	5 °C /min, repeat 3	-14.62	-7.92	117.20
Fractional points	2	5 °C /min, repeat 3	-13.01	-5.55	157.83
Membrane pore size	3	5 °C /min, repeat 3	-12.28	-5.84	145.37
1.2 µm	4	5 °C /min, repeat 3	-13.80	-6.57	156.37
	7	5 °C /min, repeat 3	-12.84	-6.52	122.53
	8	5, 2 and 1 °C /min	-14.99	-8.42	112.65
	11	10, 5 and 2 °C /min	-13.08	-6.45	188.70
	13	5 °C /min repeat 2	-12.15	-7.15	71.65
	13	10, 5 and 2 °C /min	-12.56	-6.52	145.63
	14	5 °C /min repeat 2, 2 and 10 °C /min	-11.75	-5.86	152.43
Block 2	20	10, 5 and 2 °C /min	-12.23	-5.50	167.53
Center points	22	10, 5 and 2 °C /min	-11.36	-5.30	185.27
Membrane pore size	24	10, 5 and 2 °C /min	-12.67	-5.33	180.20
0.7 µm	25	5 °C /min repeat 3	-11.93	-5.26	203.90

Oil recovered from fresh salmon waste (FW3 – FS, FW3 – RS)

Melting thermograms obtained from conventional DSC for both crude and purified oil samples of the frozen (FW3 - FS) and raw (FW3 – RS) are given in Figures 5.12 and 5.13. Three purified samples were compared with one crude oil sample. Thermograms with consistently large and wider peaks with dashed lines in Figure 5.16 are for the crude oil recovered from the frozen waste. A sharp peak with a maximum temperature at 0 °C occurred in the crude oil sample at an 8 °C/min heating rate, and a smaller peak at 2 °C /min, and no peak at 5 °C/min. Differences in peak sizes are due to heating rates and the absence of the peak at 5 °C/min is likely due to instrumental error. This peak did not occur for purified samples from the frozen waste, but occurred for crude and two purified samples from the raw waste. This is likely due to higher presence of water in crude oil from raw waste, compared to crude oil from frozen waste.

Further, as seen in Figure 5.13, thermograms for the crude sample from the raw waste also presented by the dash lines were similar or lower in area/size than for purified samples. This could be due to higher presence of impurities in crude oil from raw waste that cannot be removed using the membrane. Thermal decomposition (peaks that are inconsistent with heating rates) did not occur in the testing temperature range, indicating thermal stability below 150 °C. Purified and crude samples from both the raw and frozen wastes had another sharp peak with onset and maximum temperatures close to -12 °C and 0 °C respectively, recurring at different heating rates and, is attributed to melting of an oil-water emulsion. Almost all additional peaks had onset temperatures below 25 °C, across all purified oil samples. Table 5.18 summarizes the average onset temperatures at three heating rates (2, 5, 8 °C/min) for the melting points and several other non-melting peaks.

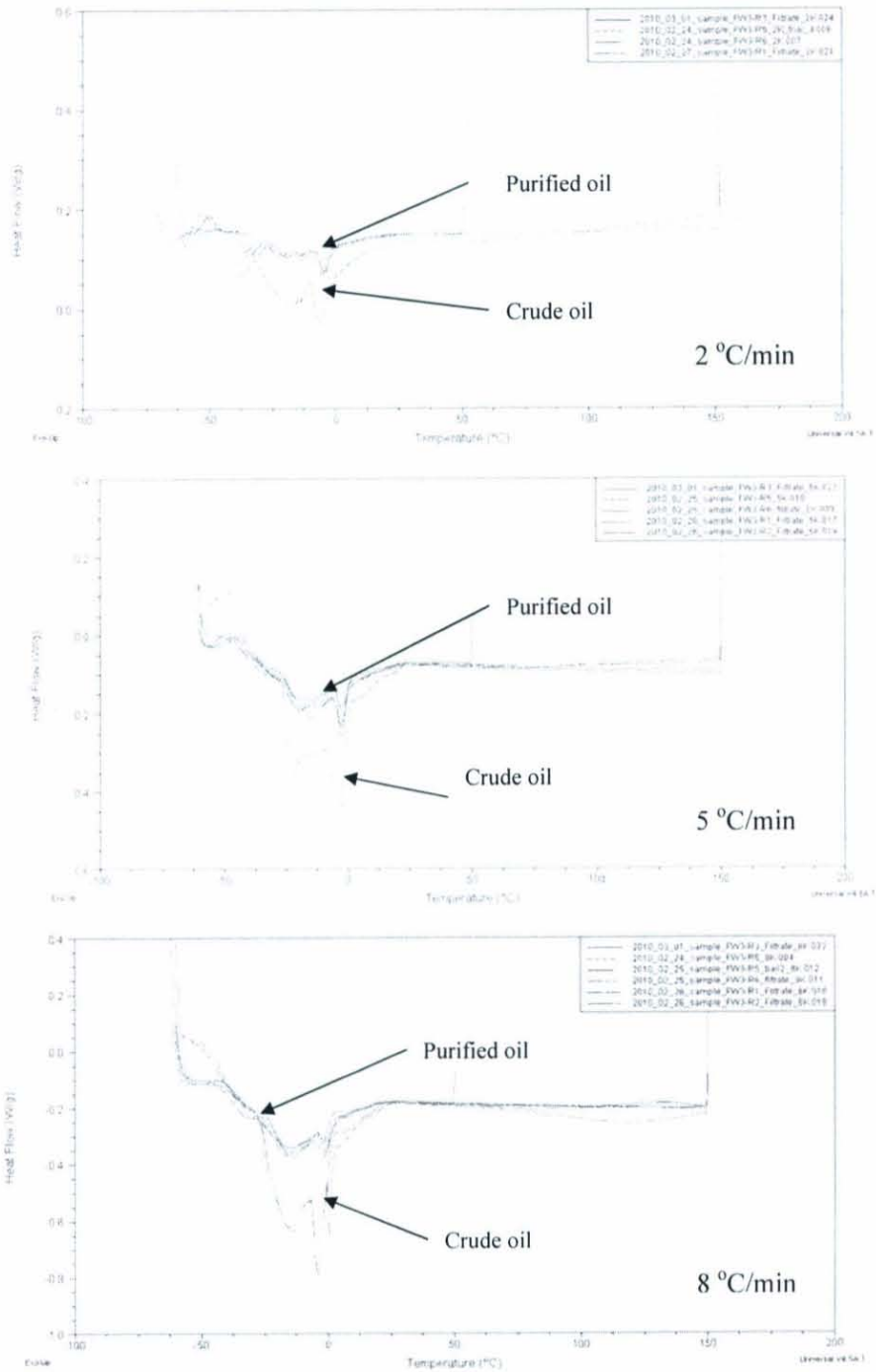


Figure 5.12: Melting thermograms for crude and purified oil from FW3 - FS

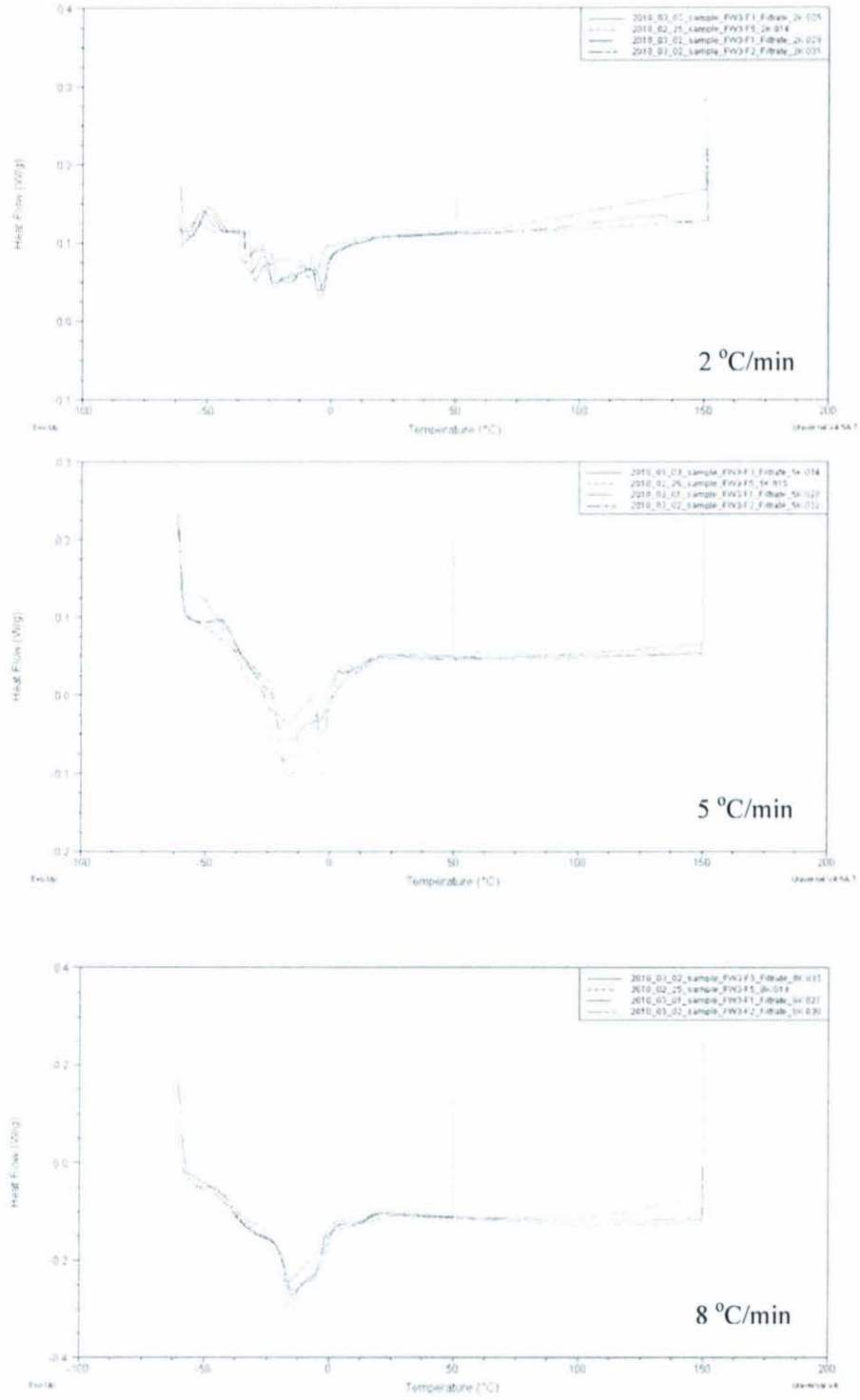


Figure 5.13: Melting thermograms for crude and purified oil from FW3 - RS

The onset temperatures of the melting points were consistent across the three heating rates. Average of the onset temperature results after analysis using DSC Thermal Analysis software and baseline optimization (after peak separation) using the AKTS software are combined for results in Table 5.18 [170]. However, only some peaks were analyzed using the latter (e.g. peak 5) due to difficulties in peak separation.

Table 5.18: Average onset melting temperature for crude and purified oil

	Average onset temperatures of the melting points (°C)						Range °C
Sample	1	2	3	4	5	6	
FS5 - crude	-38.98	-27.76	-15.64	-7.92	-0.006	3.11	-38 to 4
FS6 - purified	-36.32	-27.93	-	-5.19	-	3.11	-51 to 4
FS1 - purified	-40.51	-25.40	-16.94	-5.95	-	-	-40 to 5
FS2 - purified	-36.66	-24.93	-20.11	-5.05	-	4.2	-50 to 5
FS3 - purified	-39.30	-24.96	-16.29	-5.27	-	6.13	-49 to 7
RS5 - crude	-35.40	-23.94	-	-7.44	-0.005	13.69	-47 to 14
RS1 - purified	-40.16	-22.47	-18.88	-4.63	-	5.48	-50 to 6
RS2 - purified	-38.77	-25.58	-18.92	-7.76	-0.005	7.8	-48 to 8
RS3 - purified	-38.27	-22.42	-18.67	-6.70	-0.003	4.38	-47 to 5

The melting and decomposition temperatures for the purified oil from frozen samples occurred at lower temperatures compared with the crude oil as shown in Table 5.18. Such an improvement was not observed for raw samples. Five consistent melting points were observed across all samples, in the rages of -35 to -41 °C, -22 to -28 °C, -4 to -8 °C, -0.005 to -0.007 °C and 3 to 7 °C. The sharp melting peak near 0 °C was observed only for crude samples and R2 and R3 purified samples. This indicates the presence of water and showed that the use of a hydrophobic membrane does not remove all of the water from the raw sample purified oil. Purification results in an observed decrease in the melting range for raw samples as shown in Table 5.18. Partial removal of impurities and water are qualitatively indicated by the decrease in peak areas for frozen samples, as shown in Figure 5.12.

5.4.2 Specific heat capacity

Product from cod and salmon whole fish mixed waste (FW1 – C and FW1 – S)

The specific heat capacity calibration cell constant, using sapphire was close to one. Results using the conventional DSC are summarized in Table 5.19, for the three measurements. Some samples showed weight losses during testing, due to the use of the aluminum hermetic pan and, were not included in the estimation. Specific heat capacity plots generated using TA Instruments Specialty Library software for a representative sample (sample 1 from block 1) is shown in Figure 5.14 and other samples are shown in Figure C-1 and Figure C-2 (Appendix C).

Table 5.19: Specific heat capacity of product from FW1

Sample	Specific heat capacity kJ/kg. °C	Sample	Specific heat capacity kJ/kg. °C
1	1.821 ± 0.691	17	3.192 ± 0.151
2	2.967 ± 0.187	20	2.906 ± 0.440
3	3.171 ± 0.070	22	2.716 ± 0.284
6	3.256 ± 0.232	24	3.046 ± 0.069
8	2.987 ± 0.573		
9	2.772 ± 0.044		
11	2.932 ± 0.216		
15	3.313 ± 0.474		

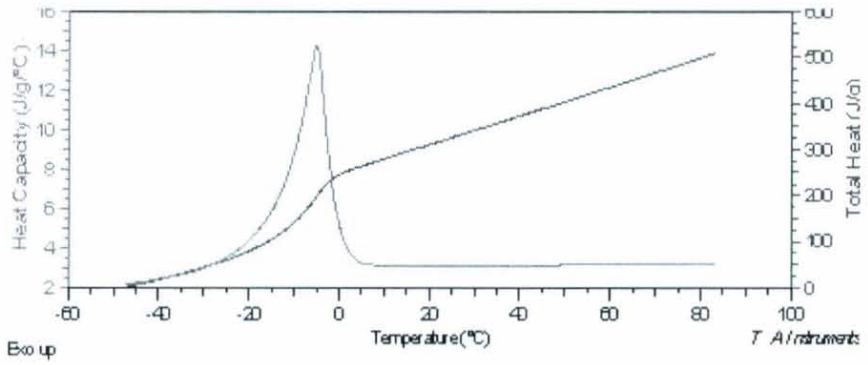


Figure 5.14: Specific heat capacity plot for samples 1, product recovered from FW1

Results from testing of samples 2 and 17 in the internally cooled DSC1 were similar to conventional DSC results. The highest value was observed for sample 15, while the lowest was for sample 1. The specific heat capacity of the product ranged from 1.8 - 3.3 kJ/kg.°C.

Oil recovered from fresh salmon waste (FW3 – FS, FW3 – RS)

The average specific heat capacity values for the purified oil recovered from frozen and raw waste samples are given in Table C-3 (Appendix C) for the three measurements. The variation in specific heat capacity with temperatures for the purified oil from raw (RS) and frozen (FS) waste samples are shown in Figures 5.15 and 5.16 respectively. The mean values and standard error of the mean (error bars for 5% confidence interval of mean) were obtained using Interval Plots in MINITAB. Specific heat capacity increased with temperatures from -50 to -20/-10 °C, after which a decrease was observed. The highest specific heat capacities for the raw samples were between -20 and -10 °C and for frozen samples at -10 °C, likely due to maximum temperature of melting/decompositions occurring at these temperatures. The average specific heat capacity values at 40 °C, for raw and frozen samples were 1.82 ± 0.37 kJ/kg.°C and 1.63 ± 0.16 kJ/kg. °C respectively, and over the entire temperature range values between raw and frozen samples were not significantly different. Specific heat capacity of the purified salmon oil from raw waste (at 20 °C) is similar values for chemically refined catfish oil (1.83 kJ/kg. °C) reported by Sathivel *et al.* (2008) [36]. Specific heat capacity values of the purified salmon oil were within range for unrefined red and pink salmon oils (0.8 – 2.3 kJ/kg.°C) [36, 133].

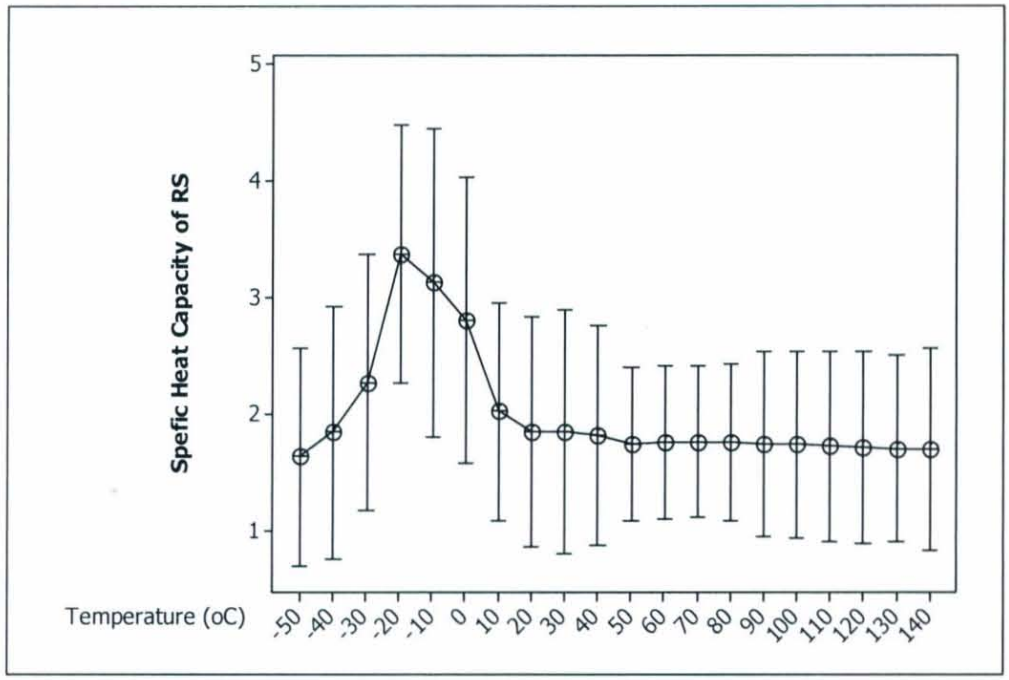


Figure 5.15: Mean specific heat capacity values of RS samples between -50 °C and 140 °C

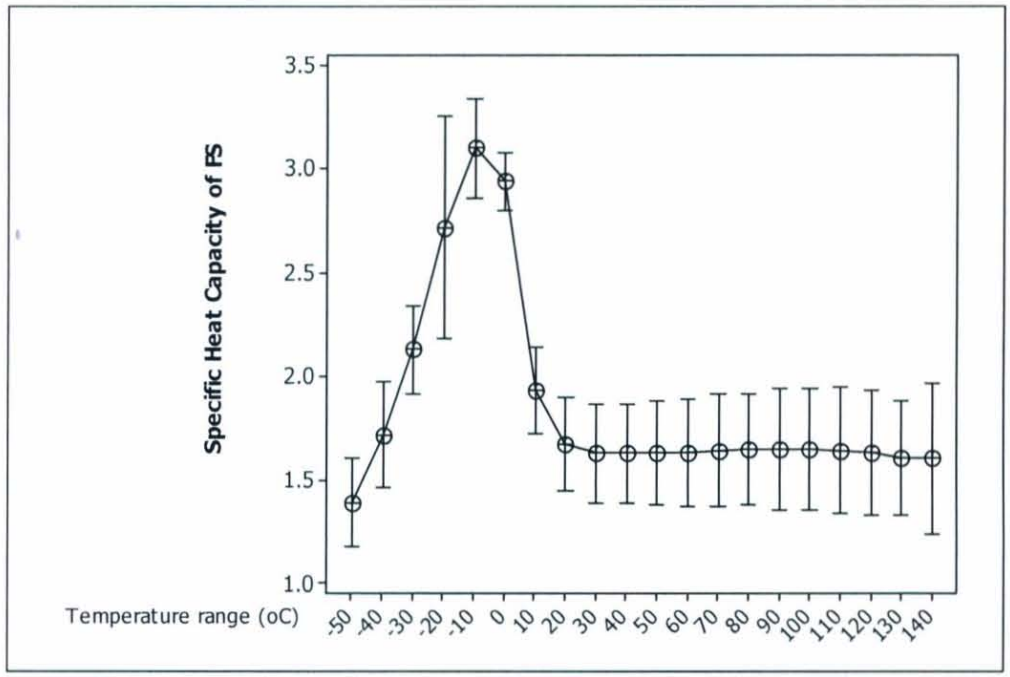


Figure 5.16: Mean specific heat capacity values of FS samples between -50 °C and 140 °C

5.5 Analysis of recovered oil: Chemical composition

5.5.1 Lipid composition

Product from cod and salmon whole fish mixed waste (FW1 – C and FW1 – S)

Analyzing the product from FW1 was difficult due to issues with drying sample spottings on the chromarods. Waiting times between spotting of 0.5 µL volumes were considerably high and a wax like layer which was difficult to remove remained on the rods. This could be attributed to higher amount of impurities or non-lipids present in the product. Developing in solvent systems did not advance the wax layer on the rods, resulting in peak areas that did not resemble the amount of sample spotted. The third scan (AMPL and PL) had no peaks and, also burns occurred on the chromarods.

Extraction of lipids from the product by initial addition of 1mL of chloroform showed a tri phase separation; a coloured (pigmented) layer on the top, a gel like white coloured layer in the middle, and a clear layer on the bottom. Addition of 2 mL of chloroform/methanol of 2:1 ratio showed a two layer separation. This procedure was successful for separating lipids from samples 1, 2, 3, 4, 10 and 13 in block 1, and for samples 22, 23 and 24 in block 2, up to the first extraction. During the second extraction, solutions from samples 22, 23 and 24 turned into a monolayer, thus additional chloroform was added. The coloured or pigmented layer was always seen at the top; however, the lipid layer was separated from the bottom. At the line of separation of the two layers, bubbles formed, likely due to proteins. Lipids extracted from the product had much less colour than lipids from the waste (FW1). This can be attributed to lower amount of lipids recovered to the product from the waste. Slight deviations in retention times between peaks in standard and samples were considered negligible.

Hydrocarbons (straight C-H chains) were present in negligible amounts across all product samples. The triacylglycerides (TAG) and free fatty acids (FFA) peaks (Figure D-1, Appendix C), were combined during iatroscanning due to the high concentrations. The manual integration function on the software was used to obtain individual peak areas. Large sterol (ST) peaks were observed for the product due to a combination of several types of ST and in some cases diglycerols. However, the exact amount of diglycerols was not quantified, as they were not part of the standard. Compared to the large wax/steryl ester (WE/SE) and ketone (KET) combined peak observed for the waste, little or no peaks were observed for the recovered product. Again, TAG and FFA peaks for both block samples (e.g. sample 2) and block 2 (e.g. sample 22) were lower than the waste. Block 1 samples had almost no TAG, while block 2 samples showed the presence of TAG. Part of the AMPL peak appeared on the end of scan 2 for some samples. Scan 3 (Figure D-3, Appendix D) did not show two single peaks for samples when compared to the standard. The presence of several types of phospholipids (PL) in fish lipids resulted in a PL peak having a combination of several peaks. Single area calculations were carried out for both acetone mobile polar lipids (AMPL) and PL peaks. The AMPL and PL peaks of the product samples 2 (block 1) and 22 (block 2) were larger compared to other lipid classes and the AMPL peak for product sample 22 was slightly larger than even for the waste. Phospholipids in general are not a desirable constituent in the use of the oil as a fuel. Samples 23 and 24 (Appendix – D), which are products from FW1 recovered in block 2 (separated using the 0.7µm acid treated membrane) had comparatively higher TAG peaks than sample 10, from block 1 as shown in Appendix – D.

Total lipid and lipid class composition of the product from FW1 are given in Table 5.20. Eight samples from block 1 (filtered using 1.2µm membrane) and three samples from block 2

(filtered using the 0.7 μm acid treated membrane) were analyzed for composition using calibration values given in Appendix A. Block 2 samples had higher total lipids than block 1 samples. Block 1 samples had little or no TAG and block 2 samples had less than 0.1 wt. % of TAG.

Table 5.20: Percentage composition of product of FW1 (wt. %)

Block	Sample No.	HC	SE	KET	TAG	FFA	ALC	ST	AMPL	PL	Total
1	1	0.001%	0.01%	0.01%	0.00%	0.00%	0.02%	0.00%	0.03%	0.05%	0.11%
	2	0.001%	0.01%	0.01%	0.00%	0.01%	0.02%	0.00%	0.03%	0.06%	0.14%
	3	0.002%	0.00%	0.01%	0.00%	0.00%	0.01%	0.00%	0.04%	0.05%	0.11%
	4	0.001%	0.00%	0.00%	0.00%	0.01%	0.02%	0.00%	0.03%	0.07%	0.13%
	5	0.001%	0.01%	0.01%	0.00%	0.00%	0.01%	0.01%	0.03%	0.03%	0.11%
	7	0.001%	0.00%	0.01%	0.00%	0.01%	0.02%	0.01%	0.04%	0.08%	0.18%
	10	0.002%	0.00%	0.01%	0.00%	0.01%	0.03%	0.01%	0.05%	0.07%	0.18%
	12	0.002%	0.00%	0.01%	0.00%	0.01%	0.02%	0.01%	0.05%	0.17%	0.28%
2	22	0.002%	0.00%	0.00%	0.06%	0.02%	0.01%	0.01%	0.08%	0.07%	0.24%
	23	0.001%	0.00%	0.01%	0.02%	0.01%	0.02%	0.00%	0.10%	0.20%	0.37%
	24	0.007%	0.00%	0.01%	0.02%	0.02%	0.07%	0.03%	0.01%	0.24%	0.49%

The highest lipid class present across all samples was phospholipids, where the composition in block 2 samples was higher than for block 1, confirming the presence of higher impurities in samples. Overall, total lipids in the product were lower than 0.5 wt. %. Wax and steryl esters were not detected in the product except for samples 1, 2 and 5, and KET was less than 0.015 wt. % across all samples. The effectiveness of the oil recovery process was determined by the percentage recovery of total and lipid classes to the oil, from the actual amount in the waste of FW1 (lipids recovered from 1 g of waste using process/ actual lipids in 1 g of waste as determined in section 5.1.6), and results are given in Table 5.21. Total lipids recovered from the waste to the block 2 product was higher than for block 1 and, negligible amount of

WE/SE and KET were recovered to both products. This could be due to effectiveness of filters. The TAG were recovered at a higher percentage to block 2 samples than block 1. Overall, the recovery of total lipids from the actual amount present in the waste (FW1) was less than 1%. This could be due to the low effectiveness of the physical separation process combined with lower amounts of lipids present in FW1. The properties of the product are not comparable to literature values due to less than or equal to 1 wt. % lipids and 78 wt. % water.

Table 5.21: Percentage recovery of lipids to the product from actual amounts in FW1

Block	Sample No.	HC	WE/SE	KET	TAG	FFA	ALC	ST	AMPL	PL	Total
1	1	0.88%	0.01%	0	0.00%	0.00%	0.66%	0.00%	0.66%	0.27%	0.07%
	2	0.98%	0.02%	0.00%	0.00%	0.31%	1.23%	0.00%	1.32%	0.63%	0.17%
	3	1.15%	0.00%	0.00%	0.00%	0.00%	0.62%	0.00%	1.17%	0.33%	0.09%
	4	0.36%	0.00%	0.00%	0.00%	0.07%	0.28%	0.00%	0.37%	0.18%	0.04%
	5	0.35%	0.00%	0.00%	0.00%	0.04%	0.42%	0.24%	0.60%	0.11%	0.06%
	7	0.30%	0.00%	0.00%	0.00%	0.10%	0.72%	0.33%	0.92%	0.41%	0.11%
	10	0.81%	0.00%	0.00%	0.00%	0.13%	0.70%	0.17%	0.73%	0.25%	0.08%
	12	0.81%	0.00%	0.00%	0.00%	0.12%	0.65%	0.19%	0.87%	0.72%	0.14%
2	22	2.70%	0.00%	0.00%	19.69%	0.83%	0.61%	0.58%	4.83%	0.99%	0.42%
	23	2.37%	0.00%	0.00%	7.25%	0.40%	2.40%	0.00%	6.90%	3.31%	0.70%
	24	13.65%	0.00%	0.00%	6.05%	0.88%	6.87%	2.17%	6.66%	3.83%	0.93%

Oil recovered from old salmon waste (FW2)

During lipid extractions, samples that were not heated during oil recovery (Ex-3 samples) had a tri phase separation from the first extraction itself and required addition of higher amounts of chloroform for clear biphase separation. This is likely due to the presence of higher amounts of proteins in the samples that were not heated (Ex-3 samples) compared to the samples that were heated (Ex-2 or Ex-1) during oil recovery. Extracted lipids were not completely representative of the oil samples as only 1 mL from top layer of recovered oil

were used for lipid extractions and, mostly bottom layer would contain water and other impurities.

Similar to the waste (FW2-S), WE/SE and KET peaks in the recovered oil were not separated and larger than the calibration interval as shown in Figures of Appendix D. The patterns of the peaks were consistently showing what could possibly be a short chain wax/steryl ester and KET, however, due to unclearness, combined peak areas were analyzed by extrapolation of calibration values given in the Appendix - B. These results indicated to wax and steryl esters and ketones between 29 – 44 wt. %, 29 – 40 wt. % and 28 – 38 wt. % in; Ex1, Ex2 and Ex3. Using the values, total lipids ranged from 54 – 61 wt. %, 52 – 60 wt. % and 52 – 56 wt. % in Ex1, Ex2 and Ex3.

Results for the percentage hydrocarbons (HC), TAG, FFA, ALC, ST AMPL and PL, by weight of the recovered oils are given in Table 5.22. Variations in measurements were higher (RSD > 50%) for TAG, ranged from 4 and 16 wt. %, and the highest was for EX3. Across all samples FFA were less than 1.5 wt. % and the highest were in EX2-1 and Ex1-1. Less than 2 wt. % of phospholipids were present in the recovered oil and hydrocarbons were the lowest lipid class present with less than 0.03 wt. %. Recovery of TAG from the old salmon waste (FW2-S) was higher compared to cold and salmon whole fish waste (FW1), indicating to effectiveness of the new oil recovery process when TAG in the waste is close to 1 wt. %.

Table 5.22: Lipid composition in the recovered oil from FW2 – S (wt. %)

Sample	No. of data	HC	TAG	FFA	ALC	ST	AMPL	PL
Ex1	4	0.02 ± 0.01	11.09 ± 2.6	0.87 ± 0.3	5.48 ± 0.7	1.52 ± 0.2	2.84 ± 0.1	1.25 ± 0.3
Ex2	4	0.02 ± 0.01	8.25 ± 2.5	0.89 ± 0.4	6.12 ± 0.7	1.65 ± 0.2	3.20 ± 0.5	1.37 ± 0.4
Ex3	3	0.02 ± 0.00	10.01 ± 5.9	0.74 ± 0.3	5.74 ± 0.5	2.04 ± 0.3	2.61 ± 0.1	1.10 ± 0.1

Relationships between the process conditions and the lipid composition values for the recovered oil were analyzed using MINITAB 14 [169]. Significant differences in TAG and total lipids recovered to the oil by centrifuging only, heating at 75 °C and heating at 100 °C were analyzed using 1-way ANOVA. Total lipids and TAG recovered for the three sets were not significantly different at both 5% (p-values of 0.431) and 10% significance levels (p-value of 0.98). The residuals in the ANOVA plots were normally, randomly and independently distributed. Heating temperature or initial separation of total liquid or oil layers did not significantly affect the recovered oil as analyzed for samples Ex1 and Ex2 using Design-Expert [163]. Therefore, since heating at a lower temperature is more economical than at a higher temperature, recovering the oil at the lower temperature can be beneficial. During the liquid/liquid extraction from the samples which are not heated or centrifuged only (Ex3) required higher amount of chloroform than the samples that were heated to recovered oil, likely due to high protein content. Therefore, heating the fish waste, at the low temperature of 75 °C, would partially remove proteins, than not heating at all (initial centrifugal separation of oil).

Purified oil from fresh salmon waste (FW3 – FS, FW3 – RS)

Except for FS – 3 from the frozen samples, rest of the purified oil samples from frozen and raw waste again showed combined peaks for wax/steryl esters (WE/SE) and ketones (KET) that were larger than the calibration interval. Diluted (1/10) samples showed separation, however, both WE/SE and KET peaks were lower in size than the calibration lower boundary, except for KET or short chain WE/SE in FS - 2, FS - 3 and RS – 2 (from raw waste). These results indicated to WE/SE and KET between 4 – 10 wt. % and 7 – 10 wt. %, in purified oil from raw and frozen waste. Triacylglyceride peaks were larger than the

calibration interval when originally extracted lipids were scanned, therefore the given results are for the diluted (1/10) samples, where peaks were within calibration interval. Deviations in peak retention times were observed for TAG, free fatty acids (FFA), alcohols (ALC) and sterols (ST), for the RS - 2 purified oil sample. Percentage lipid and lipid class composition values were obtained by analyzing the peaks using calibration values given in Appendix - A. Lipid composition of the purified oil by percentage weight of 1 g of sample is given in Tables 5.23. Large variations were observed in lipid composition of purified oil, where RSD ranged from 3 – 40% for TAG, FFA, ALC, ST and acetone mobile polar lipids (AMPL) and higher than 50% for HC, WE/SE, KET and phospholipids (PL). This could be due to variations during waste sampling, oil recovery and lipid extractions.

Table 5.23: Total lipid and lipid class compositions for the purified oil (wt. %)

wt%	HC	WE/SE + KET	TAG	FFA	ALC	ST	AMPL	PL	Total
R1	1.03%	6.41%	42.04%	3.55%	1.65%	3.21%	0.82%	0.16%	58.87%
R2	0.00%	8.13%	46.82%	1.65%	1.22%	3.14%	1.12%	0.13%	62.21%
R3	0.55%	5.60%	46.28%	2.12%	1.85%	3.41%	1.34%	0.21%	61.35%
F1	0.55%	0.00%	47.22%	1.69%	2.61%	7.99%	1.19%	0.01%	61.26%
F2	0.63%	7.13%	68.25%	2.58%	2.13%	9.49%	1.24%	0.05%	91.49%
F3	0.00%	9.32%	55.75%	3.85%	2.16%	11.27%	1.25%	0.16%	83.76%

The average percentage total lipids in the purified oil were about; 61% and 79 wt. % for purified oil from raw (FW3-R) and frozen (FW3-F) samples respectively. The highest lipid class was TAG and on average 45 to 57 wt. % were present in raw and frozen samples respectively. The purified oil from the raw waste had on average, 3 wt. % of ST while purified oil from the frozen waste had between 8 and 11 wt. %. The FFA content varied between 2 – 4 wt. % across all purified oil samples. Percentage lipids in purified oil, was used for estimating percentage recovery of lipids to the purified oil from the actual present in

the waste (Table 5.7). The average total lipid and TAG recovered to the purified oil from the waste were 41% and 43% TAG respectively.

5.5.2 Fatty Acid composition analysis

Product from cod and salmon whole fish mixed samples (FW1 – C and FW1 – S)

Product samples from the mixed whole fish waste samples were analyzed for individual and categorical fatty acids and the results are given in Table 5.24. The limited components in the standard resulted higher than 50% of percentage of the unidentified components for samples 2 and 12. Samples 4, 5, 22 and 24, no fatty acids were detected within the C14 – C20 range. For samples 2, 3 and 7; C18:2 ω6 had the highest concentration as compared to C18:1 ω9 for the fish waste (FW1), indicating to possible lipid oxidation. Sample 2 had higher PUFA, and samples 3, 4, 7 and 12 had higher amount of SFA. From the identified fatty acids, SFA were the highest across all samples, possibly due to lipid oxidation.

Table 5.24: Fatty acid composition of product from FW1 (wt. %)

	2	3	4	5	7	12	22	24
C14:0	5.70	4.11	0.00	0.00	3.27	1.84	0.00	0.00
C14:1	0.00	0.00	0.00	0.00	0.28	0.28	0.00	0.00
C15:0	0.11	0.88	0.00	0.00	0.26	0.94	0.00	0.00
C15:1	0.00	0.00	0.00	0.00	0.00	0.72	0.00	0.00
C16:0	0.74	21.61	0.00	0.00	19.08	7.92	0.00	0.00
C16:1	0.00	6.57	0.00	0.00	8.36	2.64	0.00	0.00
C17:0	0.86	8.61	0.00	0.00	0.00	0.78	0.00	0.00
C17:1	0.80	8.47	0.00	0.00	0.46	2.75	0.00	0.00
C18:0	9.17	8.29	0.00	0.00	7.02	1.34	0.00	0.00
C18:1ω9	8.99	4.04	0.00	0.00	6.81	6.01	0.00	0.00
C18:2 ω6	19.57	13.95	0.00	0.00	14.75	5.02	0.00	0.00
C18:3 ω3	0.00	0.00	0.00	0.00	1.09	0.00	0.00	0.00
C20:1	0.00	0.00	0.00	0.00	3.66	0.00	0.00	0.00
unknown	54.06	23.48	0.00	0.00	34.97	69.76	0.00	0.00
SFA	16.58	43.50	0.00	0.00	29.63	12.82	0.00	0.00
MUFA	9.79	19.07	0.00	0.00	19.56	12.41	0.00	0.00
PUFA	19.57	13.95	0.00	0.00	15.83	5.02	0.00	0.00

Purified oil from fresh salmon waste (FW3 – FS, FW3 – RS)

Representative GC graphs showing ratty acid peaks for the purified oil from the frozen (FW3-F) and raw (FW3-R) waste, obtained using Varian Galaxie Chromatography Data System (version 1.9.3.2) are presented in Figures; 5.17 and 5.18. The majority of the larger peaks were seen in the low carbon chain region between C14 and C18. A large peak was also seen just before the range of analysis. Individual and categorical fatty acid composition results are summarized in Table 5.25.

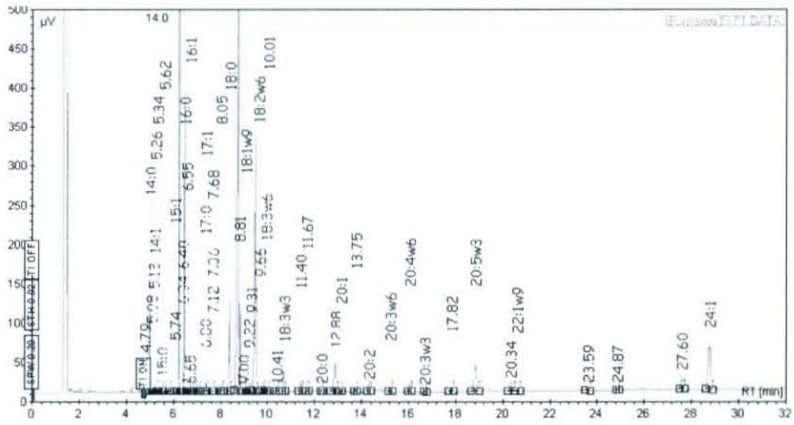


Figure 5.17: Representative GC graph for the purified oil from frozen waste (FS – 1)

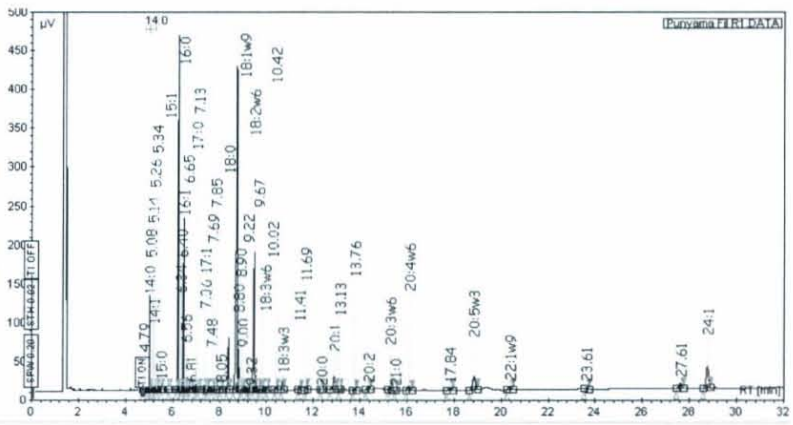


Figure 5.18: Representative GC graph for the purified oil from frozen waste (RS – 1)

Table 5.25: Fatty acid composition as a percentage by weight for the purified oil

	FS	RS
C14:0	2.78 ± 0.03	2.71 ± 0.02
C14:1	0.10 ± 0.01	0.09 ± 0.01
C15:0	0.19 ± 0.00	0.18 ± 0.00
C15:1	0.04 ± 0.00	0.03 ± 0.00
C16:0	17.01 ± 0.50	16.38 ± 0.32
C16:1	8.13 ± 0.10	8.23 ± 0.06
C17:0	0.38 ± 0.02	0.37 ± 0.02
C17:1	0.20 ± 0.08	0.43 ± 0.02
C18:0	4.40 ± 0.22	4.12 ± 0.07
C18:1ω9	32.33 ± 0.35	32.74 ± 0.10
C18:2ω6	13.03 ± 0.35	13.21 ± 0.17
C18:3ω6	0.15 ± 0.01	0.13 ± 0.02
C18:3ω3	1.06 ± 0.05	1.06 ± 0.03
C20:0	0.04 ± 0.00	0.05 ± 0.02
C20:1	2.09 ± 0.11	2.08
C20:2	0.57 ± 0.03	0.55 ± 0.03
C20:4ω6	0.75 ± 0.07	0.75 ± 0.07
C20:3ω3	0.00 ± 0.00	0.03 ± 0.01
C20:3ω6	0.43 ± 0.01	0.47 ± 0.03
C20:5ω3	3.03 ± 0.29	3.06 ± 0.15
C21:0	0.00 ± 0.00	0.00 ± 0.00
C22:0	0.00 ± 0.00	0.00 ± 0.00
C22:1ω9	0.78 ± 0.13	0.81 ± 0.001
C22:6ω3	0.00 ± 0.00	0.00 ± 0.00
C22:2	0.00 ± 0.00	0.00 ± 0.00
C23:0	0.00 ± 0.00	0.00 ± 0.00
C24:0	0.00 ± 0.00	0.00 ± 0.00
C24:1	4.54 ± 0.15	4.56 ± 0.19
Unknown	8.93 ± 1.47	9.65 ± 1.45
SFA	24.95 ± 0.48	23.81 ± 0.37
MUFA	47.26 ± 1.50	47.33 ± 1.49
PUFA	18.87 ± 0.49	19.26 ± 0.43
C14-16	28.24 ± 0.40	27.62 ± 0.27
C17-19	51.54 ± 0.21	51.97 ± 0.33
C20-22	6.75 ± 1.95	6.24 ± 1.54
C23-24	4.54 ± 0.15	4.56 ± 0.19

Oleic (C18:1ω9), C18:2ω6 and C16:0 acids had the highest concentrations in the purified oil from both raw and frozen waste. Similar to the fish waste (FW3), the purified oil showed the highest composition in monosaturated fatty acids (MUFA) and ranged between 45 and 49 wt.

%, while saturated fatty acids (SFA) were slightly higher than the polyunsaturated fatty acids (PUFA). The highest carbon number chains were 77 - 79 wt. % in the C17 - C19 range and, compared to 9 – 12 wt. % of fatty acid chains in the C20 -2 4 range. There were no significant differences (using 2- way ANOVA) in fatty acid class composition between the purified oil recovered from frozen or raw fish waste samples, however, absolute areas occupied by the fatty acids from raw samples were higher than for the frozen samples. There were also no differences, on average, between the fatty acid composition of the purified oil and fish waste (FW3 - S).

5.5.3 Elemental sulphur content

Purified oil recovered from fresh salmon waste (FW3 – FS, FW3 – RS)

Results for calibration standards and two samples each for purified oil from raw and frozen waste are given in Table 5.26.

Table 5.26: Elemental sulphur content of the purified oil

Sample	Wt. of sample / standard(g)	Total weight (g)	Initial conc (ppm)	Diluted conc (ppm)	Dilution factor	Ppm range in original sample	Average ppm in original sample	% RSD
Calibration blank 1								1.22
Standard 1 (10ppm S)	1.0073	10.002	100	10.070				2.79
0.5 ppm check	2.527	5.031	1.005	0.505			0.59	13.85
1 ppm check	**1.006**	**10.006**	**10**	**1.005**			**1.17**	3.05
5 ppm check	0.503	10.062	100	4.998			5.59	1.18
RS - 1	1.000	10.016			10.014	5.66 - 7.65	**6.58**	3.07
RS - 2	0.995	10.007			10.054	7.19 - 9.71	**8.35**	3.92
FS - 1	1.007	10.035			9.964	7.78 - 10.50	**9.04**	1.22
FS - 3	1.006	10.003			9.947	8.29 -11.19	**9.63**	3.37

The sulphur content of one of the purified oil from raw waste (R - 2) was below the detection of 1 ppm. Across all samples the RSD values were less than 4% and the purified oil from raw

samples had higher percentage sulphur than the frozen samples. The average sulphur content of the purified oil from both frozen and raw waste was 8.4 ppm.

5.5.4 Moisture content

Product from cod and salmon whole fish mixed sample (FW1 – C and FW1 – S)

The air oven method could not be used due to evaporation losses of oil and therefore a modified vacuum oven method measured both moisture and volatile matter. Use of acetone in this method served the purposes of, prevention splattering of oil during heating, dissolving both oil and water, and better evaporation of volatile matter. The boiling point of edible fish oils is about 250 °C; however, the volatile compounds present in the product interfered with obtaining the actual moisture content. Freeze drying did not prove to be a better method, due to the boiling of the oil during drying at a vacuum, resulting in inhibition of moisture evaporation. A Karl-fischer volumetric titration, as proposed in AOCS standards for fats and oils, could not be used due to unavailability of equipment [166]. Moisture and volatile matter contents for both block 1 and 2 using vacuum oven method are given in Table 5.27. Average moisture content of the product was 78 wt. %. Statistical analysis showed higher moisture and volatile matter in block 2 samples compared with block 1. The residue after heating was a solid like material, indicating the presence of high amount of waxes and impurities.

Table 5.27: Moisture and volatile matter in the product from FW1 (wt. %)

Block	Filter membrane pore size	No of samples	Moisture (wt. %)
Block 1	1.2 μm	7	77 ± 3
Block 2	0.7 μm	9	79 ± 1

Chapter 6

Discussion of Results

6.1 Fish processing effluent characteristics

The type of fish, discarded part and season of harvest impact the oil content of fish processing plant effluents as indicated by the differences in lipid composition of cod whole fish waste and salmon gut materials. Rancidity of the old salmon waste indicated that storage at a temperature 4 ^{o}C does not prevent lipid oxidation over long term (1 – 4 months). According to Bechtel *et al.* (2006), Triacylglycerdies (TAG) are the highest lipid class in fish oils [10]. However, less than 1wt. % TAG were present in both the cod/salmon whole fish waste and old salmon gut material, although the fresh salmon gut material had 45 - 56 wt. %. Higher impurities (WE/SE – wax/sterl esters, KET – ketones and PL - phospholipids) were observed in the old salmon waste and cod and salmon whole fish waste compared to fresh salmon waste, and also the free fatty acids (FFA) were higher in fresh salmon waste than other effluents. This could indicate to hydrolysis or oxidation of TAG; in salmon gut material in general during fish processing (as freshly analyzed) and cod/salmon whole fish waste likely due to storage time/temperature before analysis. Lipid oxidation in cod could have enhanced due to enzymes present in the viscera [45, 108]. The differences in TAG content in old and fresh salmon gut material, could also be due to season of fish harvest, where the old material was acquired at the end of summer and fresh during winter. Accumulation of oil in the fish is higher during the winter than the summer. Lipid impurities such as PL, KET, WE/SE and sterols (ST) can impact cold temperature properties, while, the FFA impact the stability of the recovered oil [24]. All of these were less than 3 wt. % in the by-products of salmon harvested in the winter; therefore these are a better feedstock for oil recovery.

The higher monosaturated fatty acids (MUFA) than saturated fatty acids (SFA) across all waste types can be advantages due to better cold temperature properties in the recovered oil.

The large variations in density, solids, acidity and BOD_5 within the same type of waste relates to the heterogeneous nature of fish processing plant effluents. High total suspended solids (TSS) and acidity in fresh salmon waste compared to high dissolved solids (TDS) and low acidity in both whole cod fish waste and old salmon gut material confirmed the comparatively higher oil and FFA in the fresh salmon by-products. Overall, the total solids and acidity of the effluent decreased after oil recovery, and further discussed in Chapter 7.

6.2 Fuel properties of the recovered oil

6.2.1 Physical and thermal properties

Physical and thermal properties were important to determine the impacts during processing and use of oil. The conventional DSC/QCA was useful in initial determination of melting regions, low temperature behaviours, specific heat capacity and impurities and to an extent, the thermal stability.

Physical properties

The viscosity values in literature for; crude fish oils, fish biodiesel, fish biodiesel and petroleum diesel, are summarized in Table 6.1, and the crude fish oil values ranged from 13 to 80 cP [8, 170, 171]. The physical properties of the purified salmon oil recovered from fresh salmon by-products are summarized in Table 6.2. Viscosities of the oil recovered from both frozen and raw salmon by-products are within the range for literature values of crude fish oils (Table 6.1), but, higher than for crude salmon oils which ranges from 14 to 36 cP (at

25 °C) [171]. Biodiesel standards (ASTM PS 121) specify a kinematic viscosity of 4 -1.9 cSt at 40 °C with a maximum moisture content 0.05 wt. % [55]. The viscosity of purified salmon oil is higher than both ASTM biodiesel specifications and petroleum diesel (Table 6.1).

Table 6.1: Viscosity of fish oil, fish biodiesel and diesel from literature [8, 68, 170, 171]

Method of extraction	Apparent viscosity at 25 °C (cP)	Source
Unrefined Pollock oil	40	[8]
Cat fish visceral oil	70 - 80	[170]
crude pink salmon oil	36	[171]
crude red salmon oil	32	
crude salmon oil	14	
purified salmon oil	13	
salmon biodiesel	4	
diesel (D1)	4	
B0 (Neat Diesel)	5.84	[68]
B20	6.18	
B40	6.81	
B60	7.48	
B80	8.04	
B100 (Neat Biodiesel)	8.37	

Table 6.2: Physical properties of the purified salmon oil from fresh salmon by products

Property	Temperature	Unit	Oil from raw waste	Oil from frozen waste
Density	25 °C	kg/m^3	914 ± 1	915 ± 2
Density	40 °C	kg/m^3	907 ± 0	907 ± 10
Dynamic Viscosity	25 °C	cP	52.50 ± 2.26	50.89 ± 0.99
Dynamic Viscosity	40 °C	cP	29.20 ± 1.07	29.39 ± 0.59
Kinematic Viscosity	25 °C	cSt	57 ± 2	55 ± 1
Kinematic Viscosity	40 °C	cSt	32 ± 1	32 ± 1
Specific heat capacity	40 °C	kJ/kg.°C	1.82 ± 0.38	1.63 ± 0.13
Melting point range		°C	-40 to 8	-47 to 7

According to a presentation made by Sathivel, chemical refining of the crude salmon oil decreased the viscosity by 1 cP, and conversion to a salmon biodiesel decreased the viscosity from 14 to 4 cP [171]. Purification of the crude salmon oil by membrane separation on

average decreased the viscosity by 0.5 cP; therefore, possibility exists for further improving the purified fish oil properties through transesterification.

Kinematic viscosities of No.2 fuel oil, marine diesel oil and No.6 fuel oil (Bunker C fuel) are 2.5, 3.8 and 602.2 cSt respectively [13]. Therefore, in terms of viscosity, purified salmon oil can replace No.6 fuel oil. Increasing the temperature from 25 to 40 °C resulted in 44% decrease in purified fish oil viscosity. Increasing the temperature from 25 to 40 °C decreased the viscosity of marine residual fuels RMA and RMB by 64%, and No.6 fuel oil by 89% [13, 169]. Therefore, the viscosity decreases of fish oil with temperature are less than residual and heavy fuel oils.

Densities of No.2 diesel, No.2 fuel oil and No.6 fuel oils are 830, 847 and 988 kg/m^3 respectively [13, 14]. The density of purified salmon oil was higher than the value of light fuel oil (No.2) and lower than that o f heavy fuel oil (No.6).

Thermal properties

According to the study by Sathivel *et al.* (2008), chemical refining of crude catfish oil decreased the melting range from, -46.2 and 21.2 °C to between -52.3 and 8 °C [36]. The upper limit of the melting range for crude salmon oil from raw waste decreased from 14 to 8 °C through purification and to a value similar to chemically refined catfish oil. Through purification reductions in peak sizes/areas of oil from the frozen waste occurred, indicating a decrease in enthalpies of melting resulting from removal of impurities; however the melting range did not decrease.

6.2.2 Chemical composition

The non-separation of an oil layer from cod and salmon whole fish waste, and 0 wt. % TAG and negligible fatty acids in the product indicates that lower amount of lipids as found in whole fish waste of cod/salmon cannot be effectively separated using a physical separation processes. Solvent based extractions can better recover the oil, as seen by higher lipids extracted, than physical separated however this requires an additional unit operation in the processing plant. The chemical composition of the purified salmon oil is given in Table 6.3.

Table 6.3: Chemical composition of the purified fish oil

Composition	Unit	Oil - R	Oil - F
Elemental sulphur	wt. %	0.001	0.001
Total lipid	wt. %	60.81	78.84
FFA	wt. %	2.44	2.71
TAG	wt. %	45.05	57.08
PL	wt. %	0.17	0.07
HC	wt. %	0.52	0.39
WE/SE	wt. %	1.88	0.56
KET	wt. %	4.83	4.92
SFA	wt. %	23.81	24.95
MUFA	wt. %	47.33	47.26
PUFA	wt. %	19.26	18.87
C14-16	wt. %	27.62	28.24
C17-19	wt. %	51.97	51.54
C20-22	wt. %	6.24	6.75
C23-24	wt. %	4.56	4.54

Saturated fatty acids (SFA) such as C16:0 (16 – 17 wt. %) are expected to increase the melting point however, the upper point in the melting range was below 8 °C. This is likely due to the presence of complex components than individual fatty acids. Apart from the slight changes during recovery and purification (increase of C21:0 of less than 0.5 wt. %, and decrease of C20:3ω6 in purified oil from frozen waste, decrease of C17:1 and C22:1ω9 in purified oil from raw waste), the fatty acid composition remained consistent over recovery of

oil from the waste. The percentage by volume of saturates in low sulphur diesel is 66% [172]. Less than 25 wt. % saturates in purified salmon oils could better cold temperature properties. According to a study by Canakci *et al.* (2003), the SFA composition in soybean oil and yellow grease are 16 wt. % and 39 wt. % in the C14 to C20 region, and remained similar during conversion to methyl ester [173]. The SFA in purified oil is lower than yellow grease and higher than soybean oil both in crude and methyl ester forms, therefore purified salmon oil may have better cold temperature flow properties than yellow grease.

The waxes (high carbon number saturates) can impact the cold temperature properties such as cloud and pour points [174]. The purified salmon oil had high monosaturated fatty acids (47 wt. %) and low impurities (less than 5 wt. % of FFA, WE/SE, KET and PL) making the oil more suitable for use as a fuel. High recovery of triacylglycerides (TAG - 43%) and total lipids (41%) to the oil from amounts actually present in the waste, indicates to the effectiveness of the recovery and purification process for waste with high TAG (15 – 40 wt. %).

Less than 5 wt. % FFA in the purified oil makes it a suitable feedstock for direct transesterification for fish biodiesel production without the need for acid catalyzed pre-esterification [30, 29, 112]. Less than 5 wt. % high carbon number (C20 – C24) chains indicate lower tendency to form gums. Sulphur in the purified oil is much lower than low sulphur diesel (500 ppm), which is the lowest across all petroleum fuels [16]. The composition and properties of purified oil from frozen and raw salmon by-products were on average not different; therefore, processing waste after short term cold storage is more economical than frozen storage.

Composition analysis of the oil was important as physical properties only relates to the behaviour of the oil but not the reasons behind it. Lipid and fatty acid composition determined the constituents in the oil that lead to a behaviour or fuel property (e.g. high amount of waxes increase viscosity). Overall, purified salmon oil, recovered from fish salmon by-products, can better replace No.6 fuel oil interms of density, viscosity and sulphur content.

6.2 Improvements for the oil recovery process

The flow diagram of the final recovery and purification process is given in Figure 6.1. Differences between the proposed process and the fish meal process are; reduction of heating temperature to 80 °C, eliminating the screw pressing step and replacing with a centrifugal step, reduction of centrifugal speed used for oil polishing and the addition of a membrane separation step for purification of oil. The life cycle analysis results, for the process are given in Chapter 7.

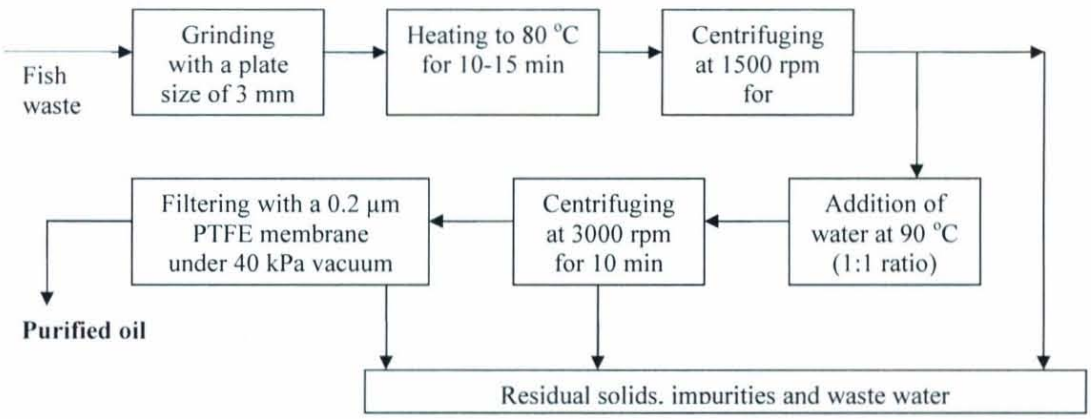


Figure 6.1: Proposed process steps and conditions for recovery and purification of fish oil

The results obtained for parameters that provide the optimum product indicates that the most important point established is the heating temperature. Heating to a low temperature of 75 °C was sufficient for recovering oil with lower impurities (proteins; however, the temperature was increased to 80 °C for uniform distribution of 75 °C in the slurry. Although fish meal plants operate at a high temperature of 90 °C, studies conducted by FAO state that rapturing of blood cells and release of oil occurs at a low temperature of 75 °C [7]. Therefore, the findings are in accordance with literature. Heating time was increased to 45 min for the separation of oil from cod/salmon whole fish waste; however for fresh salmon by-products with high TAG required heating time of only 15 to 20 min similar to the fish meal process [7]. The optimum heating temperature was common across all fish waste types tested.

The addition of water at 90 °C, followed by centrifuging at 3000 rpm for 10 min (25 °C), partially removed residual solids and water and the centrifuge time and speeds were less than literature values. Separation of water, residual solid impurities from the fish oil was achieved in several studies by centrifuging at either; 15500 x g (10 - 17 °C), 7250 x g for 30 min (23 °C), 5000 rpm (2560 x g) for 30 min or 9600 x g for 20 min (4 °C) [11, 36, 170, 171]. According to these studies below ambinent centrifuging temperatures was better in impurity separation [11]. However in the present study, addition of water at 90 °C dissolved part of the residual solids in the water resulting in the removal of solids in the oil.

The above process recovered oil with total lipids between 50% and 62%. Although pressing can aid separating a higher amount of liquid from the slurry, oil water emulsions can form inhibiting separation oil as seen for the product from cod/salmon whole fish waste. Separation of oil from waste with high total lipids and TAG as with fresh salmon by-

products, was possible even with centrifuging only. Addition of an oil polishing step as used in the fish meal industry resulted in removing some impurities, however, the ratio of water to oil which better purifies the product was not studied as a factor.

According to studies, the particle size distribution of tuna oil and sardine oil emulsions is between 0.05 and 20 μm where emulsions were created by the addition of artificial emulsifiers and homogenezing [175, 176]. Therefore, the presence of water in the products from cod/salmon whole fish waste could indicate to stable water-oil emulsions permeating through the membrane. The 0.2 μm hydrophobic PTFE membrane was effective in partial removal of non-lipid impurities as qualitatively indicated by the decrease in DSC peaks. The membrane separation process has advantages over chemical refining due to; low cost of operation, ease of implementation on-site in fish processing plants and preventing the addition of chemicals and avoiding contamination of the wastewater.

Chapter 7

Life Cycle Analysis

There are four steps in an LCA process; scope, boundary, inventory and improvement analysis. In the LCA, the use of pure and blends of fish oil with petroleum fuels was compared with traditional petroleum fuels.

7.1 Scope

The scope of this analysis included the energy consumption and emissions for on-site recovery and purification of fish oil (Figure 7.2), and on-site/in community use. The fish oil/ petroleum fuel blends compared were 100% and 50% fish biofuel blends (by weight) for furnaces and stationary diesel engine use, and 5% and 10% fish biofuel blends for residential boilers. In addition, the solid/liquid waste generated was compared. The life cycle analysis was conducted for a time frame of one year, for a remote fish processing community in NL as the geographical base. Recovering and purification of the oil were assumed to be carried out in an existing plant with fish waste generation of 1000 t/y and processing capacity of 100 kg/batch (or plant run).

7.2 Process Boundary

The process boundary for the fish oil recovery included recovery and purification of fish biofuel from the waste, and use as blend or pure in residential boiler, furnace or stationary diesel engine (Figure 7.1). The boundary included for the petroleum fuels; domestic crude oil production, transport, refining and petroleum fuels transport to NL.

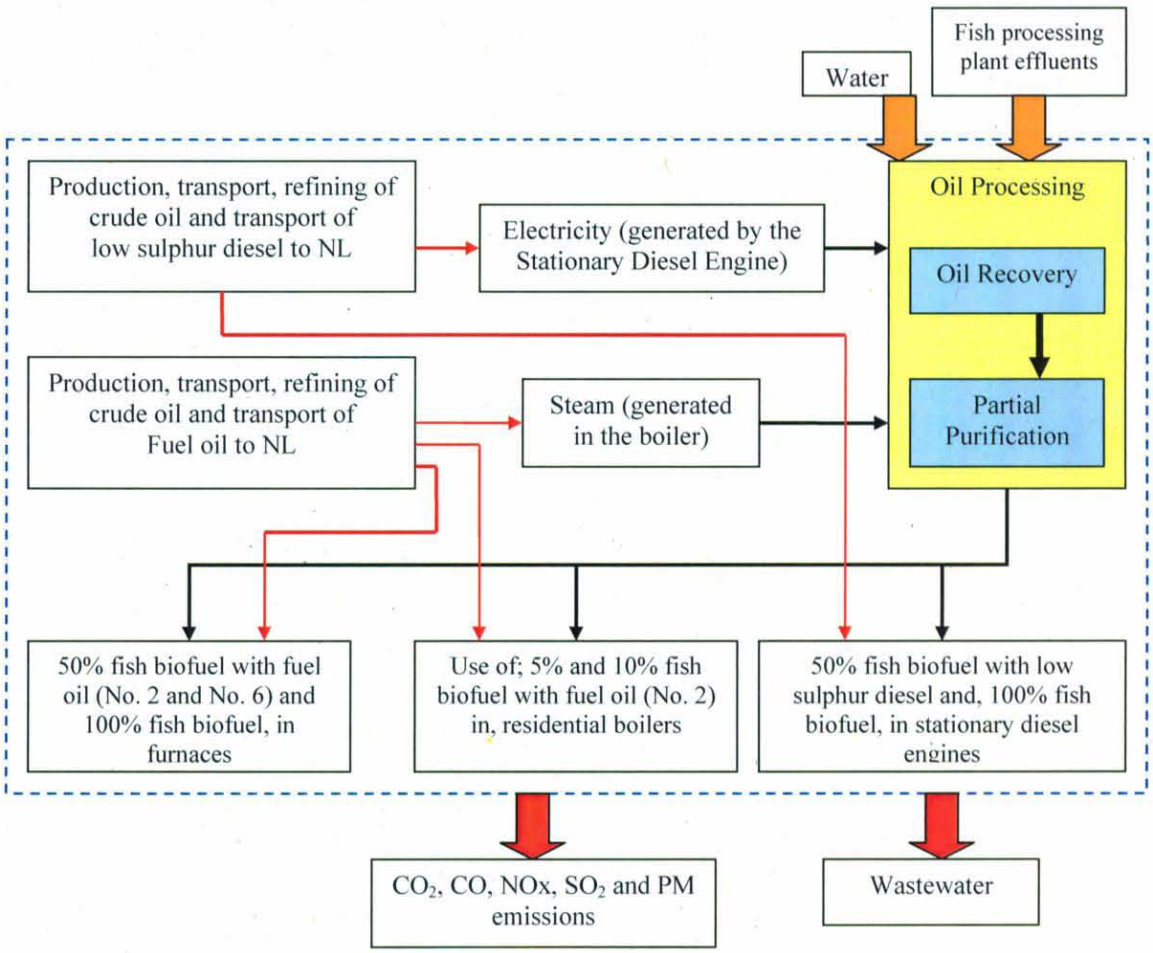


Figure 7.1: LCA process boundary

The process for recovering from the waste and purification of fish oil, is outlined in Figure 7.2, and is based on experimental results discussed in Chapter 6. Waste from the fish processing plant is stored for one to seven days in a cold storage room at the facility and, transferred to a grinder (5.2 kW Hobart type with an estimated average capacity of 75 kg/h) using a suction pump. Back pressure generated from the waste is used for transferring the ground waste directly to the heater. Based on typical heating equipment/medium in the fish meal industry, heating is carried out in a steam jacketed kettle using 80 psi steam. The fish waste is heated to 80 ^{o}C in 15 - 20 min and the slurry (containing solids, water and oil) is

pumped to a vertical centrifuge and centrifuged at 1500 rpm for 5 min. The oil is removed from the top layer, while the bottom layer containing the water and a major part of the solids is sent through the plant wastewater stream. The recovered oil and hot water at 90 °C are pumped to a second higher capacity centrifuge at an assumed ratio of 2:1 (oil: water), and centrifuged at 3000 rpm for 10 min. The bottom layer containing water and dissolved impurities are removed and discharged to the wastewater. The washed oil is sent through a PTFE membrane (0.2 μm pore size), under a 68 kPa vacuum, for purification. The purified oil (fish biofuel) is pumped to a storage tank till use and later preheated before use. When using fish biofuel blends they are pumped through a mixer or blender and mixed with petroleum fuel prior to heating.

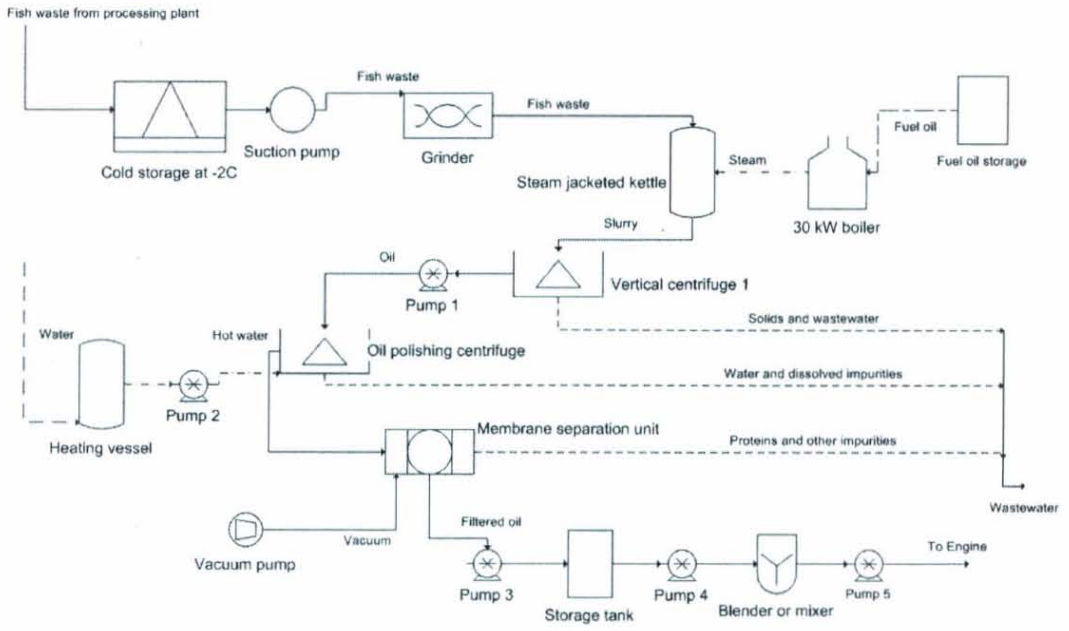


Figure 7.2: Process flow diagram for recovery and purification of fish oil

The electrical energy for the fish biofuel processing plant is assumed to be generated using an on-site stationary diesel engine operating on low sulphur diesel, and the steam is assumed to be generated in a small residential type boiler (30kW) burning No.2 fuel oil.

7.3 Inventory Analysis

Inventory analysis was conducted for gaseous and particulate emissions and solid/liquid waste discharge to the water. The types of emissions analyzed included; CO, CO_2, NOx, SO_2 and PM. The solid/liquid waste constituents were estimated in terms of acidity and solids (TS, TSS, and TDS) by using experimental results for effluents before and after the recovery of oil from Chapter 5.

Emissions associated with acquisition of petroleum fuels, acquisition (processing) of fish biofuel and use in boilers, furnaces and stationary diesel engines were included. Emissions during extraction, processing and, transport of petroleum fuels required for fish biofuel processing and use in engines were taken from a study by the NREL (1998) [16]. Energy requirements for fish biofuel processing were estimated using experimental results from Chapter 5, and estimating emissions. End-use CO_2, CO, NOx and PM emissions for; 100% fish biofuel, 50% fish biofuel with 50% low sulphur diesel and pure petroleum fuel for stationary diesel engines were taken from the study by Steigers (2003) [14]. Furnace and residential boiler (5% and 10% fish biofuel) end-use emissions (CO_2, CO, NOx and PM) were taken from Wang *et al.* (2008) [13]. The SO_2 emissions for fish biofuel were estimated using fuel sulphur content of 0.001 wt. % in fish biofuel (experimental results) and, petroleum fuel values given in Steigers (2003) and Wang *et al.* (2008) [13, 14]. Fuel properties of fish biofuel are summarized in Table 3.15.

Emissions associated with electricity and steam generation for fish biofuel processing were estimated using stationary diesel engine end-use emissions in Steigers (2003) and residential boiler values in Wang *et al.* (2008) [13, 14].

7.3.1 Inventory analysis for energy consumption during fish biofuel processing

Energy consumption values for; cold storage of fish waste, transfer to grinder, grinding, heating, centrifuging, oil polishing and membrane separation were included. In addition, energy for steam production, hot water production, pumps, pre-heating of the fish biofuel and blending were also incorporated. The percentage removal/reduction of waste during each stage of the process are outlined in Table 7.1, and were based on experimental calculations.

Table 7.1: Assumptions for the energy consumption estimation of fish biofuel processing

Unit operation	Reductions in waste	Assumptions
Cold storage	0 wt. %	- Energy consumption of a chill storage room is linearly correlated to the volume of storage - Storage volume is 25 m^3
Transfer to grinder	5 wt. %	- The volumetric suction air flow rate is equal to the flow rate of the fish waste from storage containers - Suction pump has an average efficiency of 87% [177]
Grinding	5 wt. %	- Motor is operating at 87% efficiency [177] - Energy is used only for operating the motor at constant speed
Heating	15 wt. %	- Specific heat capacity of fish waste is similar to that of water - Steam generation boiler efficiency is 85% [177] - Ambient temperature is 20 °C
Centrifuging	60 wt. %	- Centrifuge radius is 25 cm [Pilot plant observations]
Oil polishing	15 wt. %	- Hot water at 90 °C is added in the ratio of 2:1 of oil: water - All the water input is separated during centrifuging - Radius of the centrifuge is 25 cm [Pilot plant observations]
Membrane separation	10 wt. %	- Active membrane area is 10 m^2
Pumping	5 wt. %	- Hydraulic standard pumps with 87% efficiency are used [177] - Average suction heads are 2 m for pumps 1, 3, 4, 5 and 1 m for pump 2
Pre-heating	5 wt. %	- A heating coil in the storage vessel is used - Oil is heated to about 32 °C [14]
Blending	5%	- Motor (standard motors) efficiency is 87% [177] - Mixing is carried out for 15 min

Removal of water during heating was allocated as 15% based on experimental results for drying the fish waste, where, at 103 - 105 °C moisture and volatile matter were reduced by 80%. The highest removal of solid waste and water of 60% was during initial centrifuging. Oil polishing and membrane separation removed residual solids, sediment and water with some losses in oil. Total removal of impurities (water, solids, and part of the waxes /KET/ PL) was 80% from the input waste. This value is based on experiments results in Chapter 5, where percentage TAG recovery was 20 wt. % from the waste.

The waste flow rates to each unit were on a batch (plant run) basis and, were used for estimating energy consumption values. Cold storage energy consumption was estimated on an annual basis. Energy for process control was assumed to be 5% of total plant energy consumption.

Cold storage

Energy consumption for operating the cold storage room at a temperature of -2 °C was modified from data in Maidment *et al.* (2000), and Equation 7.1 [178]. The study included energy required for maintaining cold storage room at the given temperature for a given area for offsetting heat losses in air filtration, lighting and fabric/material conduction and cooling the product.

$$E_{CS} = E_{UK} \times V_{CS} / V_{UK} \qquad (7.1)$$

Where, E_{CS} is the energy consumption in the pilot plant cold storage (MJ/s), E_{UK} is the energy consumption values given in the study (MJ/s), V_{CS} is the volume of cold storage in plant (m^3), and V_{UK} is the volume of cold storage facility in the study (m^3)

Transport to grinder using a suction pump

A centrifugal suction pump within a 7.5 kW power rating was used, to operate at a vacuum of 68 kPa, and suction rate of 5.1 m^3/min, using literature values [179]. Equations 7.2 and 7.3 were used for input mass flow rate and duration of operation estimations. An average fish waste bulk density of 1531 kg/m^3 was based on experimental results. Energy for the pump was estimated as of Equation 7.4 using, power rating and duration of operation.

$$MF_{SP} = S_{SP} \times 1/60 \times D_{FW} \quad (7.2)$$

Where, MF_{SP} is the mass flow rate through the suction pump (kg/h), S_{sp} is the air suction flow rate of pump (m^3/min), and D_{FW} is the density of fish waste (kg/m^3)

$$T_{SP} = Min_{SP} / MF_{SP} \quad (7.3)$$

Where, T_{SP} is the duration of operation of the pump per run (h/run), and Min_{SP} is the mass input to pump (kg/run)

$$E_{SP} = P_{SP} \times (1/ Ef_{SP}) \times T_{SP} \times 3600 \quad (7.4)$$

Where, E_{SP} is the energy consumption in the pump (MJ/run), P_{SP} is the power rating of the pump (MW), and Ef_{SP} is the pump efficiency

Grinding

It was assumed that grinding is carried out using a pilot scale grinder (2.24 kW Hobart type). Experimental observations using a similar type of grinder, with 3 mm plates, had a grinding capacity of 75 kg/h. Energy is consumed for operating a motor at constant speed for rotation

of the screw, and the duration of operation and energy consumptions were estimated using Equations 7.5 and 7.6.

$$T_G = Min_G / C_G \quad (7.5)$$

Where, T_G is the duration of operation of the grinder (h/run), Min_G is the mass input to grinder (kg/run), and C_G is the grinding capacity (kg/h)

$$E_G = P_{GP} \times (1/ Ef_{GP}) \times T_G \times 3600 \quad (7.6)$$

Where, E_G is the energy consumption in the grinder pump (MJ/run), P_{GP} is the power rating of the pump (MW), and Ef_{GP} is the pump efficiency

Heating

Heating was assumed to be carried out in a steam jacket kettle with 100 kg capacity, using steam at 80 psi. Mixing is not carried out in the heater in order to allow better separation of oil and water. Steam was assumed to be generated in on-site boiler (30 kW) using No.2 fuel oil. Specific heat capacity of the fish waste was assumed to be similar to water, due to 80% moisture and volatile matter in the waste. Steam energy generation requirement in the boiler was estimated using Equation 7.7, by using boiler efficiency of 85% [179]. Heat losses in pipelines were assumed at 10% from what's transferred from boiler

$$E_B = Min_K \times SHC_{CFW} \times (T_F - T_{in}) * 1.25 \quad (7.7)$$

Where, E_H is the steam energy requirement in the boiler (MJ/ run), Min_K is mass input to the steam kettle (kg/run), SHC_{CFW} is specific heat capacity of fish waste or water (MJ/kg.K), T_F is the final temperature of fish waste heating (80 ^{o}C), and T_{in} is the room temperature (20 ^{o}C)

Centrifuging

According to literature data, losses in the centrifuge are about 50% due to energy dissipation as heat [180]. Power consumption in a centrifuge is calculated by the multiple of torque and angular velocity, using Equation 7.9, and the torque was estimated in Equation 7.8.

$$Tr = D_S \times FR_S \times \omega \times (R_E)^2 \quad (7.8)$$

Where, Tr is the torque (Nm), D_S = density of slurry (kg/m^3), FR_S is the volumetric flow rate of slurry (m^3/s), ω is the angular velocity of centrifuge ($rad.s^{-1}$), and R_E is the exit radius of slurry (m)

$$E_C = Min_C \times \omega \times (Min_C)^2 \times 2 \quad (7.9)$$

Where, E_C is energy consumption in the centrifuge (MJ/run), and Min_C is the mass input of slurry to the centrifuge per run (kg/run)

Oil polishing

Oil polishing requires the addition of water at 90 °C at 2:1 ratio of oil: water, and centrifuging. Heating the water was assumed to be carried out in a vessel equipped with a coil via electrical heating and the electrical energy requirement was estimated using Equation 7.10. Impurity separation was assumed to be carried out in a vertical centrifuge with a radius of 25 cm, at speed of 3000 rpm for 10 min, based on experimental results in Chapter 5. Equations 7.8 and 7.9 were used for estimating the energy, for a mass input of hot water and oil.

$$E_{OP} = (Min_C / 2) \times SHC_{HW} \times (TF_{HW} - Tin_{HW}) \quad (7.10)$$

Where, E_{OP} is the energy input to the water heater (MJ/run), Min_C is the mass of oil input to the centrifuge (kg/run), SHC_{HW} is the specific heat capacity of water (MJ/kg.K), TF_{HW} is the final temperature of hot water (^oC), and Tin_{HW} is the room temperature of water (20 ^oC)

Membrane separation

Experimental results showed an average membrane flux of 3 $kg/m^2.h$, at 68 kPa vacuum, for an active membrane diameter of 42.5 mm. These results were used for calculating pilot plant membrane flux using Equation 7.11, where the active membrane area was assumed at 10 m^2. Energy for operating the vacuum pump (7.5 kW at 5.1 m^3/min suction rate) was estimated using Equation 7.12.

$$MF_M = MF_{Mh} / A_M \quad (7.11)$$

Where, MF_M is the mass flow rate (kg/h), MF_{Mh} is the flux on the test membrane ($kg/m^2.h$), and A_M is the active area in the pilot plant membrane (m^2)

$$E_M = PR_{MVP} \times (M_M / MF_M) \quad (7.12)$$

Where, E_M is the vacuum pump energy input (MJ/run), PR_{MVP} is the pump power rating (MW), and M_M is the total mass input to the membrane (kg/run)

Pumps

Pumps are used in the plant for transferring the slurry from heater to centrifuge (Fig. 7.2, pump 1), hot water to second centrifuge (Fig. 7.2, pump 2), oil from membrane separation unit to the storage tanks (Fig. 7.2, pump 3), oil from storage to blender (Fig. 7.2, pump 4) and

blender to site of use (Fig. 7.2, pump 5). A differential head of 1 m was assumed for pump 2, as the water is pumped to the bottom of the centrifuge to avoid formation of water-oil emulsions.

$$E_{SPEP} = MF_{EP} \times GF \times DH_{EP} / Ef_{EP} \quad (7.13)$$

Where, E_{SPEP} is the energy to provide shaft power for each pump (MJ/run), MF_{EP} is the mass flow rate through each pump (kg/run), GF is the gravitational acceleration (ms^{-2}), DH_{EP} is the differential head for each pump (m), and Ef_{EP} is the pump efficiency

Preheating of recovered oil

Electrical energy for heating the oil to 32 °C, was assumed to be supplied to the heating coils in the storage tank. The required energy was estimated using Equation 7.14, with the assumption of negligible heat losses. Specific heat capacity of the recovered oil was based on average of experimental values within the 20 - 32 °C temperature range (1.63 kJ/kg.°C).

$$E_{ST} = SHC_{RC} \times Min_{ST} \times Tdif \quad (7.14)$$

Where, E_{ST} is the energy for the heating the coil (MJ/run), SHC_{RC} is the average specific heat capacity of the purified oil (MJ/kg.K), Min_{ST} is the mass input to the storage tank per run (kg/run), and Tdif is the temperature increase from 20 °C (room temperature) to 32 °C

Blending

Blending is assumed to be carried out in a vessel equipped with an agitator and the energy for operating a motor (7.5 kW), for a mixing time of 15 min was estimated using Equation 7.15.

$$E_B = T_{BM} \times PR_{BM} \quad (7.15)$$

Where, E_B is the energy for mixing in the blender (MJ/run), T_{BM} is the time of operation of the mixer (s/run), and PR_{BM} is the power rating of the motor (MW)

Total plant energy requirement

Energy per plant run was estimated using the sum of energy requirements for individual operations excluding the cold storage. Assuming the required cold storage capacity is consistent for a day and the cold storage operated all year, annual energy consumption was estimated using Equation 7.16.

$$Epy_{CS} = E_{CS} \times Ty_{CS} \quad (7.16)$$

Where, Epy_{CS} is the energy consumption in cold storage (MJ/y), and Ty_{CS} is the duration of operation (s/y)

7.3.2 Emissions for petroleum fuels

Emissions for this stage was taken from the study by NREL (1998), assuming domestic (within Canada) extraction, production and transport of petroleum fuels (No.2 and No.6 fuel oils and low sulphur diesel oil) to NL are similar to US national average values for petroleum fuels [16]. The life cycle comprised of; obtaining crude oil from an onshore drilling facility, transport, refining and, transport of required amount of petroleum fuels to the site of use. Carbon dioxide emissions were derived by deducting the tail pipe emissions from life cycle emissions as given in the NREL study.

7.3.3 Emissions from purified fish biofuel substitution to furnace

A 700 kW furnace was used as the basis and, average fuel use, stack flow rate and percentage emissions from the study by Wang *et al.* (2008) were used for estimating emission factors (EF) [13]. The CO_2 emissions were estimated by Equation 7.17 and CO, PM and NOx (NO) using Equation 7.18. The SO_2 emissions were based on formulae in the study by AGS (2007) as given in Equation 7.19 and, the average fuel sulphur in blends was estimated using Equation 7.20 [181].

$$EF_{CO2} = SF \times PE_{CO2} / FUR_F \quad (7.17)$$

Where, Em_{CO2} is the CO_2 EF (kg/kg of fuel use), SF is the average stack flow rate (kg/h), PE_{CO2} is the average percentage CO_2 emissions (%), and FUR_F is the average fuel use rate (kg/h)

$$EF_O = SF \times E_{AO} \times 0.000001 / FUR_F \quad (7.18)$$

Where, Em_O is the other (CO, PM, SO_2 and NOx) EF (kg/kg of fuel use), and E_{AO} is average emissions (ppm)

$$EF_{SDESO2} = 20 \times PS_{100\%/B} \quad (7.19)$$

Where, EF_{SDESO2} is the SO_2 EF (g/kg fuel), and PS is percentage by mass of sulphur in 100% fish biofuel or blend as used in the study (m/m %)

$$PS_B = (PS_{FO} \times PB_{FO}) + (PS_{PF} \times PB_{PF}) \quad (7.20)$$

Where, PS_B is average fuel sulphur in blend (m/m%), PS_{FO} is percentage sulphur in fish biofuel (m/m%), PB_{FO} is percentage of fish biofuel in blend (wt. %), PS_{PF} is percentage sulphur in petroleum fuel (m/m%), and PB_{PF} is percentage of petroleum fuel in blend (wt. %)

7.3.4 Emissions using purified fish biofuel in boilers

Emissions were estimated using values in Wang *et al.* (2008) for 5% and 0% fish biofuel with No.2 fuel oil in a 30 kW boiler and, 10% and 0% fish biofuel with No.2 fuel oil in a 150 kW boiler [13]. Average stack flow rates and fuel use rates in all applications were assumed to be similar to the values for the furnace operating with No.2 fuel oil. Equations 7.17, 7.18, 7.19 and 7.20 were used for estimating emissions. Particulate emissions data were available only for the 30 kW boiler in the Wang *et al.* study and was for $PM_{2.5}$ emissions [13].

7.3.5 Emissions using purified fish biofuel in stationary engines

Emissions were calculated for two types of stationary diesel engines; 2152 kW operating at 720 rpm and a 2300 kW operating at 900 rpm, for substitution of low sulphur diesel fuel with 100% and 50% fish biofuels. Fuel use in each engine was using values for fuel density in the study by Steigers (2003) and Equation 7.21 [14]. Stack flow rates were estimated by Equation 7.22, using density values for air, in literature [182, 183].

The CO_2 EF were calculated using stack flow rate values and Equation 7.23, assuming similar engine conditions as given in Steigers (2003) [14]. Density of CO_2 at the corresponding stack temperatures was taken from literature values [183]. Equation 7.19 was used for SO_2 EF, where fuel sulphur in the 50% fish biofuel blend was estimated using

Equation 7.20. Density of 50% fuel blend was derived from Equation 7.24, and heating values of neat fuels and their blends were estimated by converting the values in "btu/gallon" as given in Steigers (2003), to MJ/kg [14]. The estimated heating values were used for PM, CO and NOx emissions as given in Equation 7.25.

$$FUR_{SDE} = FU_G \times D_{LSD} \quad (7.21)$$

Where, FUR_{SDE} is the fuel use in engine in neat or blend form (kg/h), FU_G is the given fuel use value (m^3/h), and D_{LSD} is the density of the low sulphur diesel fuel used (kg/m^3)

$$SF_{SDE} = SF_G / D_{Ast} \quad (7.22)$$

Where, SF_{SDE} is the stack flow rate (m^3/h), SF_G is the average stack flow rate in (kg/h), and D_{Ast} is the extrapolated density of air at the stack temperature (kg/m^3)

$$EF_{SDECO2} = SF_{SDE} \times Em_V \times D_{CO2st} / FUR_{SDE} \quad (7.23)$$

Where, Em_{SDECO2} is the CO_2 EF (kg/kg of fuel use), Em_V is the percent volumetric emissions (v/v%), and D_{CO2st} is the density of CO_2 at stack temperature (kg/m^3)

$$D_B = (D_{FO} \times PB_{FO}) + (D_{PF} \times PB_{PF}) \quad (7.24)$$

Where, D_B is average density blend (kg/m^3), D_{FO} is density of fish biofuel (kg/m^3), and D_{PF} is density of petroleum fuel (kg/m^3)

$$EF_{SDEO} = Em_{GSDE} / D_F \quad (7.25)$$

Where, EF_{SDEO} is the EF of PM/CO/NOx (kg/kg of fuel), EF_{GSDE} is the EF as given in the study (kg/m^3) and D_F is the density of fuel (kg/m^3)

7.3.6 Life cycle emissions from pure or blend of fish biofuel in engines

Energy for operation of engine/combustor per year was calculated by Equation 7.26, using power rating values and assuming a capacity factor of 0.8 and efficiency of 0.85. These energy requirements (per year) were assumed to be supplied using fish biofuel/petroleum fuel blends of 100%, 50% and 0% in stationary diesel engines and furnaces and 5% and 10% in boilers. These were calculated using Equations 7.27 and 7.28 with the respective heating values and where heating values were not available, they were calculated with Equation 7.29.

$$Ey_{E/C} = PR_{E/C} \times T_{E/C} \quad (7.26)$$

Where, $Ey_{E/C}$ is energy requirement per year for engine (MJ/y), $PR_{E/C}$ is power rating (MW), and $T_{E/C}$ is the duration of operation per year (s)

$$FUy_{E/C} = TEy_{E/C} / CV_F \quad (7.27)$$

Where, $FUy_{E/C}$ is the pure fish biofuel or petroleum fuel needed per year (t/y), $TEy_{E/C}$ is total energy needed per year (MJ/y), and CV_F is heating value of neat fuel (MJ/t)

$$FUy_{BE/C} = TEy_{E/C} \times P_{FO/PF} / CV_B \quad (7.28)$$

Where, $FUy_{BE/C}$ is fish biofuel or petroleum fuel needed per year in for blend (t/y), $P_{FO/PF}$ is percentage of fish biofuel or petroleum fuel in blend (wt. %), and CV_B is heating value of blend (MJ/t)

$$CV_B = (CV_{PF} \times PB_{PF}) + (CV_{FO} \times PB_{FO}) \quad (7.29)$$

Where, CV_{PF} is heating value of petroleum fuel (MJ/t), and CV_{FO} is heating value of fish biofuel (MJ/t)

Life cycle emissions associated with extraction, transport to processing the fuel blends were calculated using Equations 7.30, 7.31 and 7.32. Energy for producing the fish biofuels was calculated with Equations 7.33, 7.34, 7.35 and 7.36, and these values were used for estimating emissions associated with use in boilers, furnaces and stationary diesel engines (Equations 7.37 and 7.38). Life cycle emissions during acquisition of petroleum fuels for use in blend and fish biofuel production were calculated using Equation 7.39 and values in section 7.2.2.

$$LCE_{FO100\%} = Em_{IUFO} + Em_{SFO} + Em_{APF} \quad (7.30)$$

Where, $LCE_{FO100\%}$ is life cycle emissions for 100% fish biofuel production (t/y), Em_{IUFO} is in-use emissions in 100% fish biofuel in engine/furnace (t/y), Em_{SFO} is emissions in the boiler and stationary diesel engine during fish biofuel production (t/y), and Em_{APF} is emissions associated with acquiring the required amount of petroleum fuels for fish biofuel production (t/y)

$$LCE_{B} = Em_{IUFO} + Em_{SFO} + Em_{APF} + Em_{IUB} + Em_{APFB} \quad (7.31)$$

Where, LCE_{B} is life cycle emissions for production of blend (t/y), Em_{IUB} is In-use emissions in use of 50% fish biofuel in engine/combustor (t/y), and Em_{APFB} is emissions associated with acquiring petroleum fuel for the use in engine/combustor itself (t/y)

$$LCE_{PF100\%} = Em_{IUPF} + Em_{APF100\%} \quad (7.32)$$

Where, $LCE_{PF100\%}$ is life cycle emissions for 100% petroleum fuel production (t/y), Em_{IUPF} is emissions during use of 100% petroleum fuel in engine (t/y), and $Em_{APF100\%}$ is emissions during acquisition of petroleum fuels for use in engine (t/y)

$$Em_{FO110\%} = (E_{FO1t} \times Uy_{FO}) + E_{SFO} \quad (7.33)$$

Where, $Em_{FO110\%}$ is electrical energy for producing 100% fish biofuel (MJ/y), E_{FO1t} is electrical energy for producing 1 t of fish biofuel (MJ/t), Uy_{FO} is fish biofuel use per year in each engine (t/y), and E_{SFO} is energy for storage of fish waste (t/y)

$$EE_{FOB} = (E_{FO1t} \times Uy_{FOB}) + EE_{BFO} + E_{SFO} \quad (7.34)$$

Where, EE_{FOB} is electrical energy for producing a blend of fish biofuel with petroleum fuel (MJ/y), EE_{BFO} is electrical energy for blending of fish biofuel with petroleum fuel (MJ/y), and Uy_{FOB} is required amount of fish biofuel in blend (t/y)

$$BE_{FO100\%} = BE_{FO1t} \times Uy_{FO} \quad (7.35)$$

Where, $BE_{FO100\%}$ is boiler energy for producing 100% fish biofuel (MJ/y), and BE_{FO1t} is boiler energy for producing 1 t of fish biofuel (MJ/t)

$$BE_{FOB} = BE_{FO1t} \times Uy_{FOB} \quad (7.36)$$

Where, BE_{FOB} is boiler energy for producing a blend of fish biofuel with petroleum fuel (MJ/t)

$$Em_{IUEEFOP} = (EE_{FOB} + EE_{FO100\%}) \times Em_{IUSDE} \quad (7.37)$$

Where, $Em_{IUEEFOP}$ is In-use emissions for obtaining the electrical energy for blend/ neat of fish biofuel production (t/t), and Em_{IUSDE} is In-use emissions from the stationary diesel engine per 1 MJ of energy production (t/MJ)

$$Em_{IUBEFOP} = (BE_{FOB} + BE_{FO100\%}) \times Em_{IUB} \quad (7.38)$$

Where, $Em_{IUBEFOP}$ is In-use emissions for acquiring steam for pure/blend of fish biofuel production (t/y), and Em_{IUB} is In-use emissions from boiler per 1 MJ of energy production (t/MJ)

$$Em_{APF(B+FOP)} = (E_{BPF} + BE_{FOB} + BE_{FO100\%} + EE_{FOB} + EE_{FO100\%}) \times Em_{APF1MJ} \quad (7.39)$$

Where, $Em_{APF(B+FOP)}$ is emissions associated with acquiring the petroleum fuel for fish biofuel production or for petroleum fuel in blend (t/y), E_{BPF} is petroleum energy required in blend (MJ/y), and Em_{APF1MJ} is emissions for acquiring of 1MJ of petroleum fuel (t/MJ)

7.3.7 Discharge of solid/liquid waste to water

Experimental results for characterization of fish processing plant wastewater and effluents from the oil processing plant (as proposed above) were used for TS, TSS, TDS, TVS, acid value and pH. Results for the fish waste were based on analysis of FW1 - C, FW2-S, FW3, however wastewater after processing is based on FW3 samples. Comparison of results before and after the process was conducted under a 5% significance level using non-parametric Mann Whitney test (Minitab 15).

7.4 Inventory analysis results and discussion

7.4.1 Oil processing plant energy requirement

Inventory results for unit operations per plant run are given in Table 7.2. Oil production in the plant is 20.13 kg/run for fish waste input of 100kg. Total electrical energy requirement for producing crude fish biofuel is 2150 MJ/t of oil, and when using the 50% blend, the value is increased to 2533 MJ/t. Boiler energy requirement for producing steam for the plant is

1876 MJ/t of oil. In addition, energy is required in the cooling storage of 3148 MJ/y. Assuming the plant has an annual fish biofuel production of 1000 t, the total plant energy requirement is 4029 GJ/y.

Table 7.2: Mass and energy balances of unit operations in fish biofuel production

Unit operation	Mass input (kg/run)	Mass output (kg/run)	Time of operation (h)	Energy input (MJ/run)
Cooling storage	100	100	8760 /yr	3148.18 /yr
Transport to grinder	100	95	0.0002	0.007
Grinding	95	90	1.27	8.75
Heating	90	77	0.33	37.75
Centrifuging	77	31	0.08	0.24
Water washing	15	20 (water and impurities)		4.49
Centrifuging (oil)	31	26	0.17	0.57
Membrane separation	26	23	0.87	23.35
Storage	24	22		0
Pre-heating	22	21		0.44
Blending	21	20	0.25	7.72
Pumping				3.36
Process control				4.33

7.4.2 Emissions for acquisition of petroleum fuels

Emission values for acquisition of petroleum values are given in Table 7.3 [16].

Table 7.3: Life cycle emissions in acquisition of petroleum fuels [16]

Life cycle stage/ Emissions (X 10^{-8} t/MJ)	CO_2	CO	NOx	PM_{10}	PM (Unspecified)	SO_2
Crude oil production		0.23	0.89	0.01	0.63	4.79
Crude oil transport		0.04	0.25	0.01	0.18	0.41
Crude oil purification		1.61	4.84	0.05	2.95	16.41
Petroleum fuel transport		0.25	0.82	0.01	0.22	0.36
Total acquisition	4666	2.13	6.80	0.01	3.98	21.97

7.4.3 Emission factors for fish biofuel use in engines

Emission factors for 0%, 50% and 100% fish biofuel in a furnace are given in Table 7.4, and, Tables 7.5 and 7.6 summarize the EF for boilers and stationary diesel engines. Emissions in boilers are for 0%, 5% and 10% fish biofuel use.

Table 7.4: Emission factors for fish biofuel and blends use in the furnace

	CO_2 (kg/kg of fuel)	CO (kg/kg of fuel)	PM (kg/kg of fuel)	SO_2 (kg/kg of fuel)	NOx(NO) (kg/kg of fuel)
No.6 fuel oil	**2.94**	0.0006	0.093	0.006	0.004
No.2 fuel oil	**2.7**	0.0004	-	**0.004**	0.003
50% fish biofuel and No.6 fuel oil	2.32	**0.0007**	0.025	0.003	**0.004**
50% fish biofuel and No.2 fuel oil	2.43	0.0003	-	0.002	0.003
100% fish biofuel	2.23	0.0002	-	**0.00002**	0.002

Table 7.5: Emission factors for substitution of fish biofuel in boilers

	CO_2 (kg/kg of fuel)	CO (kg/kg of fuel)	PM (kg/kg of fuel)	SO_2 (kg/kg of fuel)	NOx(NO) (kg/kg of fuel)
30 kW boiler					
No. 2 fuel oil	**3.49**	0.0005	**0.018**	**0.004**	**0.003**
5% fish biofuel and No.6 fuel oil	3.02	0.0005	0.017	0.004	0.002
150 kW boiler					
No. 2 fuel oil	**3.49**	**0.0006**	-	0.004	0.003
10% fish biofuel and No. 2 fuel oil	3.02	0.0005	-	0.004	0.002

Table 7.6: Emission factors for substitution of fish biofuel in stationary diesel engines

	CO_2 (kg/kg of fuel)	CO (kg/kg of fuel)	PM (kg/kg of fuel)	SO_2 (kg/kg of fuel)	NOx(NO) (kg/kg of fuel)
2152 kW engine operating at 720 rpm					
LSD	**1.67**	**0.004**	**0.0012**	**0.001**	**0.061**
50% fish biofuel and LSD	1.4	0.003	0.0006	0.0005	0.052
100% fish biofuel	1.22	0.002	0.0003	0.00002	0.047
2300 kW engine operating at 900 rpm					
LSD	**1.84**	**0.002**	**0.0009**	**0.001**	**0.051**
50% fish biofuel and LSD	1.51	0.001	0.0006	0.0005	0.042
100% fish biofuel	1.29	0.001	0.0005	0.00002	0.038

LSD – Low Sulphur diesel

7.4.4 Life cycle emissions for pure or blend of fish biofuel in engines

Emissions in engines for each life cycle stage are given in Tables 7.7, 7.8, and 7.9. Furnace 1 and 2 are 700 kW, and use No.6 and No.2 fuel oils respectively. Boiler 1 is a 30 kW boiler using No.2 fuel oil and 5% fish biofuel as the blend, and Boiler 2 is rated at 150 kW and uses No.2 fuel oil and 10% fish biofuel as the blend. Stationary diesel engines (SDE) 1 and 2 use low sulphur diesel, where SDE 1 is 2152 kW and operates at 720 rpm, and SDE 2 is 2300 kW and operates at 900 rpm.

Table 7.7: Life cycle emissions in Furnace 1 and Furnace 2

t/y	CO_2	CO	PM	SO_2	NOx (NO)
Furnace 1					
100% No.6 fuel oil	795	0.24	15.26	2.45	1.11
50% fish biofuel and No.6 fuel oil	593	0.23	4.40	1.38	1.25
100% fish biofuel	454	0.10	0.16	0.19	1.00
Furnace 2					
100% No.6 fuel oil	734	0.21	0.29	2.16	0.88
50% fish biofuel and No.6 fuel oil	598	0.16	0.23	1.24	0.99
100% fish biofuel	452	0.10	0.15	0.19	1.00

Table 7.8: Life cycle emissions in Boiler 1 and Boiler 2

t/y	CO_2	CO	PM	SO_2	NOx (NO)
	Boiler 1				
100% No.6 fuel oil	36.6	0.01	0.13	0.09	0.04
5% fish biofuel and No.6 fuel oil	33.5	0.01	0.12	0.09	0.04
100% fish biofuel	-	-	-	-	-
	Boiler 2				
100% No.6 fuel oil	183	0.05	0.06	0.47	0.19
10% fish biofuel and No.6 fuel oil	165	0.05	0.06	0.44	0.18
100% fish biofuel	-	-	-	-	-

Table 7.9: Life cycle emissions in SDE 1 and SDE 2

t/y	CO_2	CO	PM	SO_2	NOx (NO)
	SDE 1				
100% No.6 fuel oil	1787	2.52	1.43	5.15	30.45
50% fish biofuel and No.6 fuel oil	1343	1.76	1.00	3.04	27.77
100% fish biofuel	864	1.42	0.66	0.59	26.78
	SDE 2				
100% No.6 fuel oil	1992	1.41	1.43	5.15	27.61
50% fish biofuel and No.6 fuel oil	1492	1.08	1.00	3.04	24.45
100% fish biofuel	965	0.85	0.66	0.59	23.72

Life cycle emissions by type of emission are compared in Figures 7.3, 7.4, 7.5, 7.6 and 7.7.

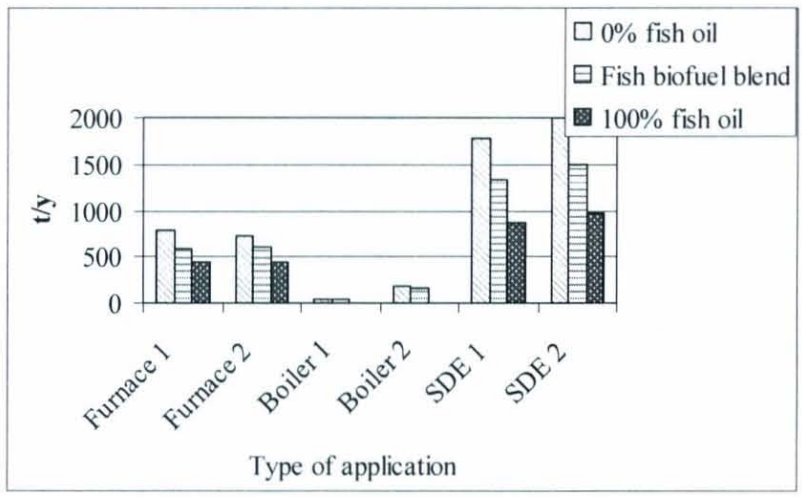


Figure 7.3: Life cycle CO_2 emissions

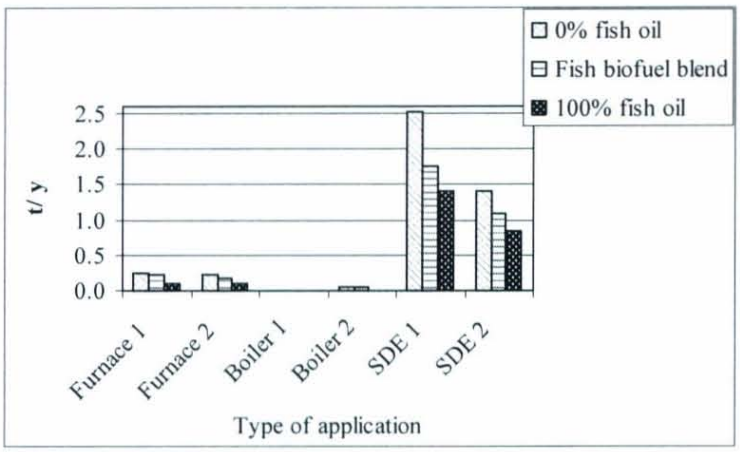


Figure 7.4: Life cycle CO emissions

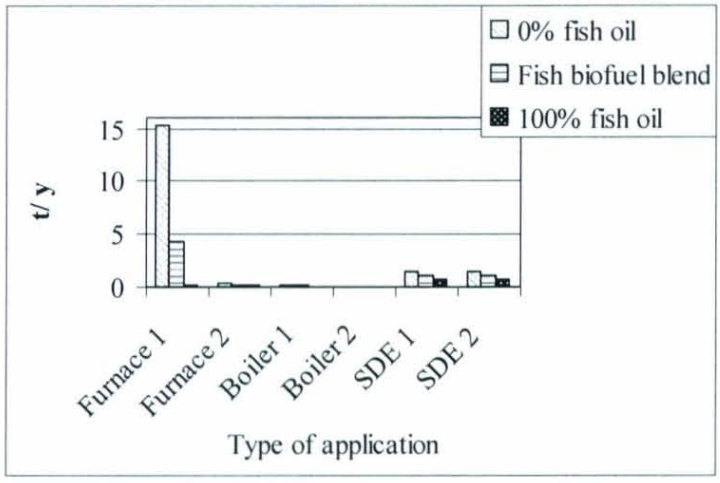


Figure 7.5: Life cycle PM emissions

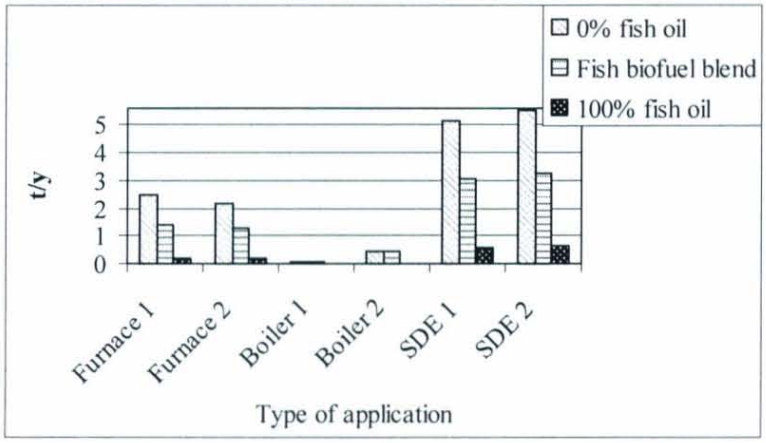


Figure 7.6: Life cycle SO_2 emissions

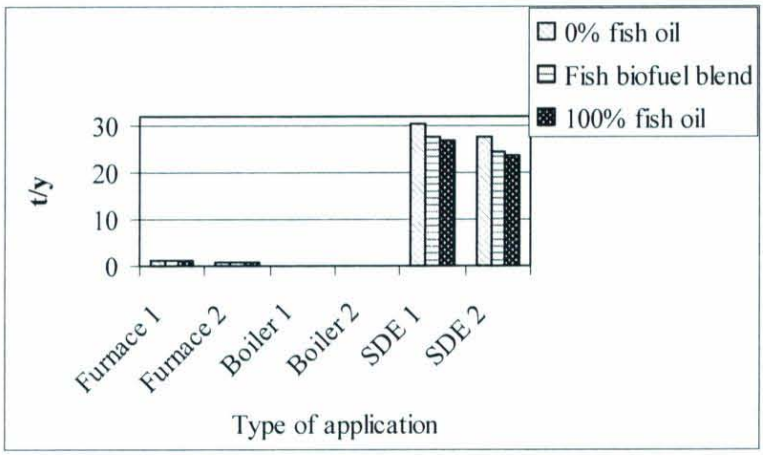


Figure 7.7: Life cycle NOx emissions

Emission reductions due to substitution of petroleum fuels with 100% fish and blends of fish biofuel are given in Table 7.10. The highest reductions are in SO_2 and CO_2, and except for NOx emissions in the furnaces and boiler 1, all other emission types are reduced. Increase in NOx emissions when using pure fish biofuel or blends varied between 6 and 13%, when compared to respective petroleum fuels.

Table 7.10: Air emission reductions in substitution of pure or blend of fish biofuel in engines

		Furnace 1	Furnace 2	Boiler 1	Boiler 2	SDE 1	SDE 2
CO_2	Fish oil blend	-25%	-19%	-8%	-10%	-25%	-25%
	100% fish oil	**-43%**	**-38%**			**-52%**	**-52%**
CO	Fish oil blend	-5%	-23%	-2%	-10%	-30%	-23%
	100% fish oil	**-59%**	**-54%**			**-44%**	**-40%**
PM	Fish oil blend	-71%	-20%	-3%	-3%	-30%	-23%
	100% fish oil	**-99%**	**-46%**			**-54%**	**-44%**
SO_2	Fish oil blend	-44%	-43%	-3%	-6%	-41%	-41%
	100% fish oil	**-92%**	**-91%**			**-89%**	**-89%**
NOx	Fish oil blend	**12%**	**11%**	**6%**	-3%	-9%	-11%
	100% fish oil	-10%	**13%**			-12%	-14%

The NOx form due to the lower volatility of fish biofuels and blends (compared to petroleum fuels) resulting in fuel oxygen combining with nitrogen in combustion air. Use of fish biofuels in higher capacity engines (e.g. stationary diesel engines with 2152 – 2300 kW) has reduced NOx emissions compared to smaller engines (e.g. boiler/furnaces <150 kW).

7.4.5 Discharge of contaminants to water

Wastewater before and after oil recovery, including the related reductions are given in Table 7.11. Statistical analysis of the results showed that recovering the oil from the fish waste resulted in significantly lowering acidity, pH, total solids (TS) and total suspended solids (TSS); however, total dissolved solids (TDS) and total volatile solids (TVS) have increased. Increase in TDS and TVS is possibly due to concentration of the non oil components of the waste, after recovering oil.

The uncertainties associated with the inventory analysis can be reduced by using experimental data for actual energy consumption in fish biofuel processing, and engine emissions for the above recovered fish biofuel.

Table 7.11: Wastewater characteristics before and after recovering the oil from fish waste

	Unit	Fish waste	Wastewater from process	Reductions
pH		6.16 ± 0.05	6.29 ± 0.05	+2.1%
Acid value	NaOH mg/ g of sample	88.47 ± 0.02	35.81 ± 0.01	-59.5%
TS	mg/L	1,121,143 - 1,334,553	233,076 - 293,149	-78.6%
TSS	mg/L	234,490 – 280,511	103,260 – 105, 398	-59.5%
TDS	mg/L	75,389 – 133,479	109,072 – 126,725	+12.9%
TVS	% by weight of sample	0.42 ± 0.17	0.87 ± 0.16	+107.1%

7.5 Impact assessment

7.5.1 Impact assessment for air

Emission types such as CO, SO_2, NOx and PM can cause ground level ozone formation, acid deposition and particulates haze [31]. Impacts from these pollutants include increased cancer risk, chronic and acute respiratory problems, asthma attacks, and visibility impairment. The life cycle analysis shows that substitution of fish biofuels in engines decrease overall emissions except for NOx. Even with 5% and 10% of fish biofuel blends in boilers, emission reductions were observed across all types except NOx.

Fine particulates as emitted through burning fuel oils in boilers and furnaces can cause acute and chronic health issues [31]. Particulate emissions are both mutagenic and carcinogenic. Particulate emissions decreased through substitution of fish biofuels across all systems.

According to the studies by Arvanitoyannis *et al.* (2008) and Garcia-Sanda *et al.* (2003), cooking fish waste in fishmeal plants and some processing plants resulted in hydrogen sulfide and trimethylamine ($(CH_3)_3N$) emissions [2, 44]. Smoke and PM emissions can also result due to heating of fish waste.

7.5.2 Impact assessment to water

Discharge of fish waste to the marine environment can cause impacts to the pelagic, sediment and benthic layer. The high organic matter present in processing effluents can cause an increase in microbial decay, which reduces oxygen concentration [185]. High oil and grease content and solids in the effluent can enhance the inhibition of direct oxygenation and light penetration to the water column resulting in suboxic and even anoxic conditions in the

shallow waters over time [184]. Visible surface slicks, turbidity plumes and attraction of undesirable species such as sharks are some of the other impacts due to organic matter [13]. Partially decomposed organic matter can add other pathogens and harmful substances to the ecosystem [185]. Over time, the particles can settle to the sediment causing changes in the sediment. Therefore, reductions in TSS, TS after recovery of oil in wastewater can reduce some of the above implications.

Toxic and carcinogenic substances are generally not present in these wastes [13]. However, the high nutrient loading on the surface water can also increase growth of toxic algal booms decreasing fish mortality [1, 185]. Proteins in fish waste contain nitrogen which can cause eutrophication, excessive phytoplankton and macro-algal growth [1]. Eutrophication promotes shifts in zooplankton and phytoplankton growth productivity and size [185]. Microalgae and filamentous algae propagation can affect benthic fauna, nursery fish and fish feeding, recreational uses and also tourism can also become affected. Anaerobic decomposition of proteins and other nitrogen compounds can result in ammonia, hydrogen sulfide and methane emissions [1, 185]. Even at low concentrations the above substances can be toxic to marine species [185]. The wastewater from the oil recovery process could be high in nitrogen as the proteins and impurities separate into the water phase during membrane separation.

When the same environment receives waste from multiple processors throughout an extended period of time there is no time for the recovery of the eco system, leading to long term impacts [185]. Some of these impacts are; reduction in species diversity in the benthic and fish communities, reduction in phytoplankton, and a increase of biomass. Overall, the

reduction of solids and acidity after oil recovery results in a reduction of environmental impacts.

The process of fish biofuel production as proposed, does not involve chemicals during recovery or purification; therefore, additional chemical contaminants are not present in the effluents. Recovering the oil reduces chemicals present in the waste such as triacylglycerides (TAG), alcohols, phospholipds (PL), wax/steryl esters (WE/SE) and sterols (ST). The oil recovery and partial purification process can reduce the overall contaminant loading on discharge site, thereby improving the conditions for marine species.

7.5.3 Other environmental impacts reduction

WISE (2004) reported biodiesel as less hazardous compared to diesel, when discharged to waterways [31]. Biodiesel was, in fact, used as a clean up agent for ground and marine diesel spills. WISE (2004) reported a study conducted by university of Idaho which showed that it takes 28 days for standard B95 to degrade, B20 degrades twice as fast compared to neat diesel, while, petroleum based fuels take several years to degrade. Therefore, use of fish biofuels and blends can be beneficial compared to petroleum fuels, due to higher biodegradability. The aquatic toxicity of diesel fuels at 96 h exposure time is highly toxic according to WISE (2004), while biodiesel toxicity was insignificant (LC_{50} > 1000mg/l) [31]. Due to the high toxicity, handling of petroleum fuels also requires HAZMAT training, while biodiesel handling does not require special training. Additionally, the flash point of neat fish biofuel is over 100^0C according to both Wang *et al.* (2008) and Steigers (2003) [13, 14]. Therefore dangerous fumes are not expected to form from fish biofuels, provided the stability is maintained without the lipid oxidation of the oil (rancid).

Corrosion of pipelines and storage tanks are expected as a result of the residual moisture present in the fish biofuels; however, with proper purification and constructions, they can be avoided. Storage of petroleum fuels typically requires multi-walled containers, whereas single walled containers are adequate for biodiesel storage, reducing the energy, materials and costs [31].

Overall, the sustainability of local communities can be improved by recovering the oil from the fish waste and substitution to on-site/in community engines and/or combustors.

7.4 Improvement assessment

The most energy intensive unit operations during fish biofuel production are membrane separation and heating. Possible solutions to reducing energy use are; improving the membrane flux and use of higher efficiency cookers such as contherm cookers. Utilizing part of the waste heat from the cold storage/ refrigeration unit to, fulfill pilot plant heat requirements in the boiler and water heating can also reduce plant energy use in fish biofuel production.

Deodorizing or suction of the plant air, scrubbing and treating the sucked air either by burning in a steam boiler or treating with chlorine has been proposed by FAO (1986), for reducing plant odour [7]. Experimental observations showed that processing of the fresh fish waste to remove oil resulted in preventing oxidation (or rancidity), and therefore reduced the odour.

The focus of this study was estimating reductions in gaseous and PM emissions and, impacts to water; implications in small engines due to long term crude fuel oil use were not studied. Further, the purified fish oils can still have contaminants that can cause the oil to freeze at near 0 ^{o}C temperatures. Transesterfication of the fish biofuel can address these issues, but may result in reduced environmental benefits due to increased chemical use, increased energy requirements and the decrease in emissions.

Chapter 8

Conclusions and Recommendations

8.1 Conclusions

The main goal of the research was to determine the feasibility and impacts of using fish waste derived biofuel for on-site and/or in-community applications in NL. The experimental study areas included; characterization of effluents from fish processing plants in NL, determining the recoverable oil, characterization of the oil, and the degree of processing for end-uses. Finally, a life cycle analysis was conducted to estimate reductions in gaseous/particulate emissions and solid/liquid waste resulting from recovery of the oil and use in engines.

Fresh salmon waste containing parts (or by-products from processing) tends to be higher in total lipids and triacylglycerides and lower in impurities (ketones, wax/steryl esters, phospholipids), than waste containing whole fish (in this study cod) or aged waste parts. The separation of oil using the proposed physical/thermal process is not feasible for waste with low oil content (4 wt. %) such as cod whole fish waste, due to water-oil emulsions. The partitioning of the oil in salmon by-products is possible through gravity separation but may contain high levels of impurities, and separation requires more processing. The fish waste has low oxidation and thermal stability as indicated by the high monosaturated fatty acids, making them susceptible to lipid oxidation; therefore, fresh waste has to be processed after a maximum of 1 week storage at 1 - 4 ^{o}C. Freezing the waste at -26 ^{o}C is not necessary over short term storage, but required for long term (> 1 week) storage.

The purified oil from fresh salmon by-products has a viscosity and density higher than diesel fuels but lower than heavy fuel oil. The oil's viscosity can be reduced by agitation, due to the "pseudo plastic" behaviour. The melting point of the purified oil was higher than petroleum fuels, but close to chemically refined fish oil. The purified oil has low oxidation stability (due high monounsaturated and saturated fatty acids), but was shown to be thermally stable at temperatures below 150 °C. An oil with high lipid (60 – 90 wt. %) and triacylglycerides (40 – 70%) content, and low impurities (on average, 10% ketones and wax/steryl esters, 4% free fatty acids) is recovered by physical/thermal separation and purification alone.

A modified fish meal process of heating to 80 °C, centrifuging instead of screw pressing, and membrane separation can recover oil which can likely replace No.6 fuel oil in terms of viscosity and density. This process can also be used as a pretreatment of oil for direct base-catalyzed transesterification, and possibly replace acid catalyzed pretreatment for feedstock with high free fatty acids (>5%). The process can recover high amount of lipids (15 – 50%) and triacylglycerides (41%) for fresh salmon by-products. Membrane separation is better than chemical refining due to similar improvement in fuel properties, lower energy use and lower effluent discharge.

The life cycle analysis of the process for reductions in gaseous/GHG emissions and solid/liquid waste indicated the proposed oil recovery and purification process can lower the impacts to waterways due to reductions in 60% acidity, 60% suspended solids, 79% total solids and 34% oil, and 19% bulk density of the discharged waste. Recovering the oil, purification, and blending in part (50%) or full substitution of the purified oil for fuel oils in furnaces and diesel fuels in stationary diesel engines can reduce life cycle emissions by 19 –

52% CO_2, 5 – 59% CO, 20 – 99% PM, and 41 - 92% SO_2. The NOx emissions increased by 6 – 13% in boilers and furnaces, but decreased by 9 -14% in stationary diesel engines. Use in high capacity engines (>2MW) can reduce NOx emissions. The emissions can be further reduced by use of fish biofuels during steam and electricity generation in the processing plant. Petroleum fuel use can be reduced by 172 – 174 t/y of fuel oil in furnaces, 535 – 572 t/y of low sulphur diesel fuel in stationary diesel engines, by pure fish biofuel use. Overall, the sustainability of rural community in NL increased due to improvements in the atmospheric and coastal water conditions.

8.2 Recommendations for future research

A study needs to be conducted on the effectiveness of membrane separation in removing lipid impurities such as wax/steryl esters, phospholipids, ketones, sterols and proteins and moisture, by analyzing the oil before and after the separation. Methods of improving the membrane flux should be studied, if the effectiveness is proven,. Alternatives for impurity separation such as; other membranes types and use of active clay or such packed beds need to be investigated. An additional literature review should focus on the effect of each lipid classes on fuel properties and the recommended reductions.

The purified oil needs analysis for moisture and protein contents, and thermal/oxidation stabilities. Standard Karl Fischer Titration method is best for moisture testing, and TGA is best for stability tests. The enthalpies of individual melting peaks obtained in the DSC can be determined by using the baseline optimization and peak separation functions in the AKTS software. The undetermined lipid classes in the wax/steryl esters and ketone regions would require confirmation by running short chain steryl ester standards. A complete ASTM

analysis of fuel properties such as boiling range, heating value, pour point, cloud point, flash point, ash content, elemental composition (C, H, N, O analysis similar to that of S) and acid value is essential in the next phase of research. These results have to be compared with ASTM standards for biodiesel and petroleum fuels and vegetable biodiesel properties in literature. The improvements in properties by blending with petroleum fuels needs research, to determine optimal blends. This will determine the degree of processing and use.

Life cycle emission studies are needed using actual data for the purified oil. The second phase of the research should study the long term implications in engines, improving the cold temperature flow, and optimal storage conditions before and after processing, needs investigation. The final phase would be transesterification of the purified oil and conducting an entire feasibility, fuel property, engine performance and, life cycle emission and solid/liquid waste analysis for the fish biodiesel. Additionally, an economic analysis of the process would determine the financial sustainability aspects.

Appendix A

Calibration Results for the Lipid Analysis

The first order linear regression equations and R-sq for lipids in FW1 – C/S and FW2 - S are presented in Table A-1, and Table A-2 presents the equations for FW3 - RS and FW3 - FS.

Table A-1: First order linear regression R-sq values and equations for lipids in FW1 – C/S and FW2 - S wastes and recovered products

Lipid class	Rack 5623			Rack 5624		
	No. of data	R-sq	Equations	No. of data	R-sq	Equations
HC	8	0.99	Y = 0.23 X – 0.10	5	0.98	Y = 0.24 X - 0.09
SE/WE	8	0.98	Y = 6.95 X – 1.93	6	0.97	Y = 0.21 X – 0.33
KET	8	0.98	Y = 0.23 X + 0.02	6	0.97	Y = 0.23 X + 0.51
TAG	8	0.95	Y = 0.45 X – 0.98	5	0.98	Y = 0.20 X + 0.48
FFA	8	0.98	Y = 0.14 X + 0.43	6	0.84	Y = 0.42 X + 0.26
ALC	8	0.99	Y = 0.27 X + 0.11	6	0.98	Y = 0.28 X + 0.37
ST	8	0.99	Y = 0.15 X + 0.34	6	0.97	Y = 0.16 X + 0.49
AMPL	8	0.99	Y = 0.20 X + 0.35	7	0.98	Y = 0.27 X + 0.25
PL	8	0.99	Y = 0.16 X + 0.83	7	0.96	Y = 0.20 X – 0.40

Y – Amount of lipid class on chromarod

X - Peak area for each rod

Table A-2: First order linear regression R-sq values and equations for lipids in FW3 - RS and FW3 - FS wastes and recovered purified oil

Lipid class	Rack 5042			Rack 5045		
	No of data	R-sq	Equations	No of data	R-sq	Equations
HC	7	0.96	Y = 0.23 X + 0.11	5	0.99	Y = 0.22 X + 0.01
SE/WE	7	0.98	Y = 6.95 X – 1.93	7	0.98	Y = 0.21 X + 0.30
KET	7	0.99	Y = 0.24 X + 0.44	7	0.97	Y = 0.25 X + 0.37
TAG	7	0.99	Y = 0.21 X + 0.52	5	0.99	Y = 0.22 X + 0.33
FFA	7	0.94	Y = 0.71 X – 0.22	6	0.97	Y = 0.42 X + 0.19
ALC	7	0.99	Y = 0.22 X + 0.40	6	0.98	Y = 0.32 X + 0.28
ST	7	0.97	Y = 0.14 X + 0.54	6	0.98	Y = 0.18 X + 0.43
AMPL	7	0.94	Y = 0.13 X + 1.05	7	0.99	Y = 0.25 X + 0.41
PL	7	0.96	Y = 0.15 X + 0.25	7	0.98	Y = 0.20 X – 0.40

Y – Amount of lipid class on chromarod

X - Peak area for each rod

The equations illustrate the correlation between the lipid concentration spotted on the rods and the resulting peak areas. The results were used in developing second order linear regressions equations for analyzing peaks larger than the calibration upper limit. Table A-3 outline regressions results used for FW1 – C/S and FW2 - S, and Table A-4 results were used for FW3 - RS and FW3 - FS.

Table A-3: Second order linear regression R-sq values and equations for analyzing lipids in FW1 – C/S and FW2 - S waste and recovered products

Lipid class	Rack 5623			Rack 5624		
	No of data	R-sq	Regression equations	No of data	R-sq	Regression equations
HC	8	0.99	$Y = 0.00 X^2 + 0.22 X - 0.08$	5	0.98	$Y = 0.01 X^2 + 0.12 X + 0.20$
SE/WE	8	0.98	$Y = -0.00 X^2 + 0.29 X + 0.28$	6	0.97	$Y = 0.01 X^2 + 0.13 X + 0.44$
KET	8	0.98	$Y = 0.03 X^2 + 0.22 X + 0.10$	6	0.97	$Y = 0.00 X^2 + 0.16 X + 0.71$
TAG	8	0.95	$Y = -0.02 X^2 + 0.78 X - 1.16$	5	0.98	$Y = -0.00 X^2 + 0.22 X + 0.42$
FFA	8	0.98	$Y = 0.00 X^2 + 0.14 X + 0.44$	6	0.84	$Y = -0.01 X^2 + 0.96 X - 0.31$
ALC	8	0.99	$Y = 0.00 X^2 + 0.22 X + 0.23$	6	0.98	$Y = 0.00 X^2 + 0.27 X + 0.37$
ST	8	0.99	$Y = -0.00 X^2 + 0.18 X + 0.23$	6	0.97	$Y = -0.00 X^2 + 0.26 X + 0.18$
AMPL	8	0.99	$Y = -0.00 X^2 + 0.24 X + 0.15$	7	0.98	$Y = 0.00 X^2 + 0.25 X + 0.30$
PL	8	0.99	$Y = -0.00 X^2 + 0.26 X + 0.07$	7	0.96	$Y = 0.00 X^2 + 0.13 X + 0.04$

Table A-4: Second order linear regression R-sq values and equations for analyzing lipids in FW3 - RS and FW3 - FS waste and recovered purified oils

Lipid class	Rack 5042			Rack 5045		
	No of data	R-sq	Regression equations	No of data	R- sq	Regression equations
HC	7	0.98	$Y = 0.01 X^2 + 0.12 X + 0.36$	5	0.99	$Y = -0.00 X^2 + 0.25 X - 0.09$
WE/SE	7	0.99	$Y = -0.00 X^2 + 0.22 X + 0.26$	7	0.99	$Y = 0.00 X^2 + 0.18 X + 0.37$
KET	7	0.99	$Y = 0.00 X^2 + 0.20 X + 0.60$	7	0.99	$Y = 0.00 X^2 + 0.15 X + 0.73$
TAG	7	0.99	$Y = -0.00 X^2 + 0.23 X + 0.46$	5	0.98	$Y = 0.00 X^2 + 0.20 X + 0.47$
FFA	7	0.98	$Y = 0.14 X^2 + 0.04 X + 0.40$	6	0.97	$Y = -0.00 X^2 + 0.48 X + 0.13$
ALC	7	0.99	$Y = -0.00 X^2 + 0.27 X + 0.32$	6	0.99	$Y = 0.02 X^2 + 0.20 X + 0.42$
ST	7	0.99	$Y = -0.00 X^2 + 0.22 X + 0.35$	6	0.99	$Y = 0.00 X^2 + 0.14 X + 0.50$
AMPL	7	0.96	$Y = -0.00 X^2 + 0.21 X + 0.65$	7	0.99	$Y = -0.00 X^2 + 0.31 X + 0.14$
PL	7	0.96	$Y = 0.00 X^2 + 0.12 X + 0.43$	7	0.98	$Y = 0.00 X^2 + 0.20 X - 0.34$

Appendix B

Statistical Analysis Results for Product Recovery of FW1

The percentage product recovery results cod and salmon whole fish mixed sample (FW1) for the designed experiment are given in the Table B-1, including the factors. The factors with the highest contribution to percentage product recovery were identified using Design-Expert software, and are presented in the Table B-2. The highest contributions were from the interaction effect between agitation and centrifuge speed (AC), the interaction effect between heating time and heating temperature (DE), the interaction effect between agitation and heating temperature (AE), heating time (D), centrifuge time (B), interaction effect between agitation and heating time (AD), and agitation (A).

Table B-1: Run combination and results for the fractional factorial experiment

Sample	Factor A: Agitation	Factor B: Centrifuge time (min)	Factor C : Centrifuge speed (rpm)	Factor D: Heating Time (min)	Factor E: Heating temperature (^{o}C)	Product recovery (wt. %)
1	Mixing	3	1500	30	90	6.92
2	Grinding	5	1500	45	75	13.35
3	Grinding	3	1500	45	90	8.39
4	Mixing	5	1500	30	75	3.28
5	Grinding	5	2500	45	90	5.54
6	Grinding	5	1500	30	90	8.64
7	Grinding	3	1500	30	75	6.49
8	Mixing	5	2500	45	75	9.40
9	Mixing	3	2500	45	90	8.11
10	Mixing	3	1500	45	75	4.83
11	Mixing	5	2500	30	90	11.96
12	Grinding	5	2500	30	75	5.40
13	Mixing	3	2500	30	75	4.24
14	Grinding	3	2500	30	90	5.48
15	Grinding	3	2500	45	75	8.58
16	Mixing	5	1500	45	90	5.56

Table B-2: Effect and Percentage contribution of Factors and interactions

Term	Effect	SumSqr	% Contribution
A-Agitation	0.945	3.572	3.148
B-Centrifuge time	1.261	6.359	5.604
C-Centrifuge speed	0.157	0.099	0.087
D-Heating time	1.418	8.040	7.085
E-Heating temperature	0.632	1.595	1.406
AB	-0.262	0.275	0.243
AC	-3.123	39.012	34.379
AD	1.043	4.351	3.834
AE	-2.074	17.207	15.164
BC	0.213	0.181	0.160
BD	-0.277	0.306	0.270
BE	-0.560	1.254	1.105
CD	-0.279	0.312	0.275
CE	0.240	0.231	0.203
DE	-2.769	30.680	27.037

The statistical significance of the chosen effects were analyzed by the Pareto chart (Figure B-1) and 1-way ANOVA (Table B-3), using Design-Expert software. The Pareto chart showed the above selected effects (AC, DE, AE, D, B, AD, and A) as the highest contributing effects. The ANOVA results for the probability of the contribution from the above selected factor and the prediction of percentage product recovery using a model that combines the factors are given in Table B-3. Excluding centrifuge speed (C), the model and the selected factors were significant (<5%). Since interaction effects of C (AC) significantly contributed to the model, C was included in the model. Individual and interaction effects that did not occur in the model were used for estimating the model error. The main assumptions behind constructing the ANOVA results were; normal distribution, random distribution and constant variance of residuals. The residual plots (Design-Expert) showed the validity of the assumptions.

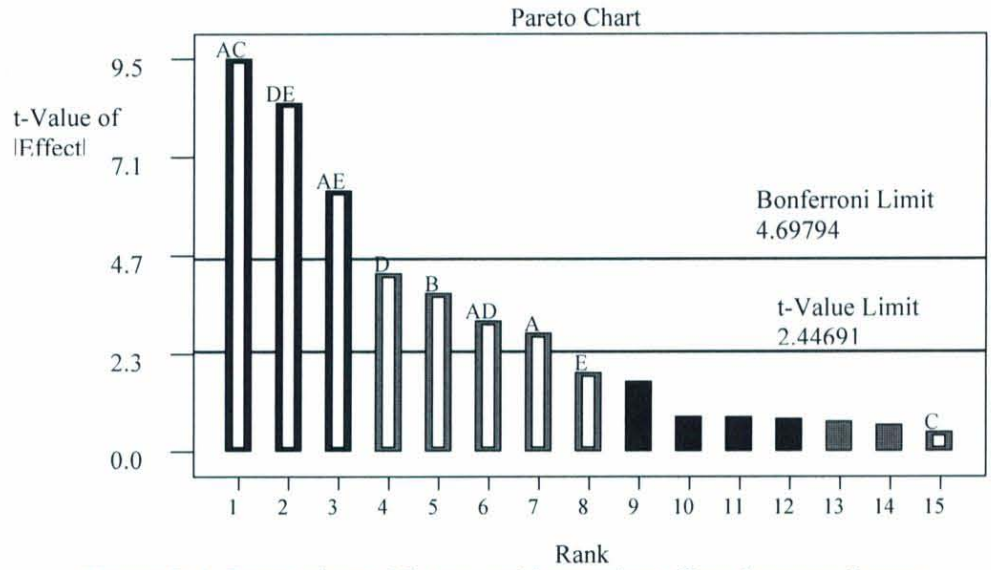


Figure B-1: Pareto chart of factors and interaction effects between factors

Table B-3: ANOVA results of the selected model factors

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	110.91	9	12.32	28.88	0.00	significant
A-Agitation	3.57	1	3.57	8.37	0.03	
B-Centrifuge time	6.36	1	6.36	14.90	0.01	
C-Centrifuge speed	0.1	1	0.1	0.23	0.65	
D-Heating time	8.04	1	8.04	18.84	0.00	
E-Heating temperature	1.6	1	1.6	3.74	0.1	
AC	39.01	1	39.01	91.42	< 0.0001	
AD	4.35	1	4.35	10.2	0.02	
AE	17.21	1	17.21	40.32	0.00	
DE	30.68	1	30.68	71.9	0.00	
Residual	2.56	6	0.43			
Cor Total	113.48	15				

Overall, the method of agitation, centrifuge time, centrifuge speed, the interaction effect of method of agitation and centrifuge speed, agitation and heating time, agitation and heating temperature and heating time and heating temperature all impact the percentage product recovered from FW1. The impact of interaction effects were investigated using interaction plots (Design-Expert), and are illustrated in Figure B-2 (heating time and temperature),

Figures B-3 (method of agitation and heating time), Figure B-4 (method of agitation and centrifuge speed), and Figure B-5 (method of agitation and heating temperature).

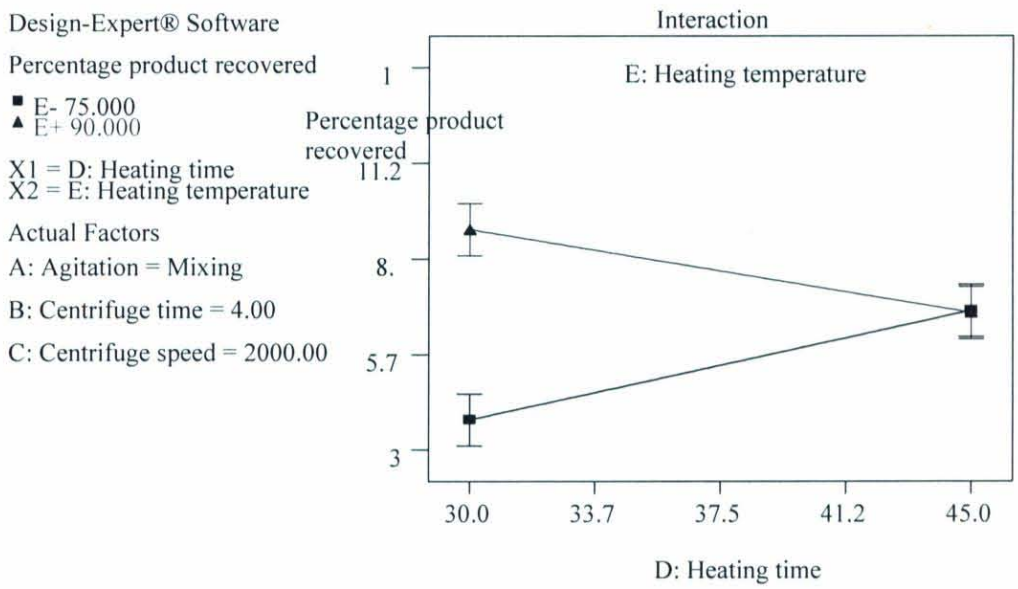


Figure B-2: Effect of the interaction between Heating time and Heating temperature

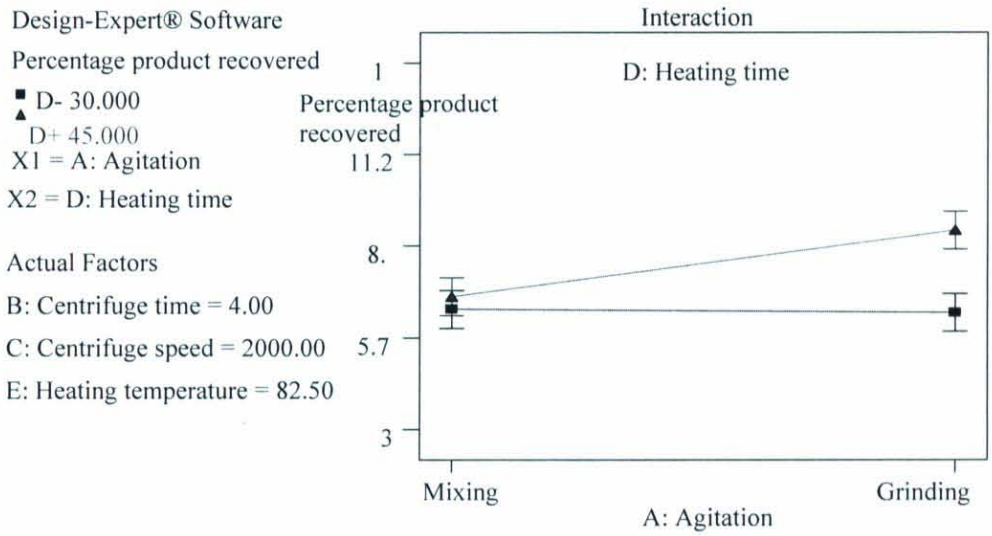


Figure B-3: Effect of the interaction between method of agitation and heating time

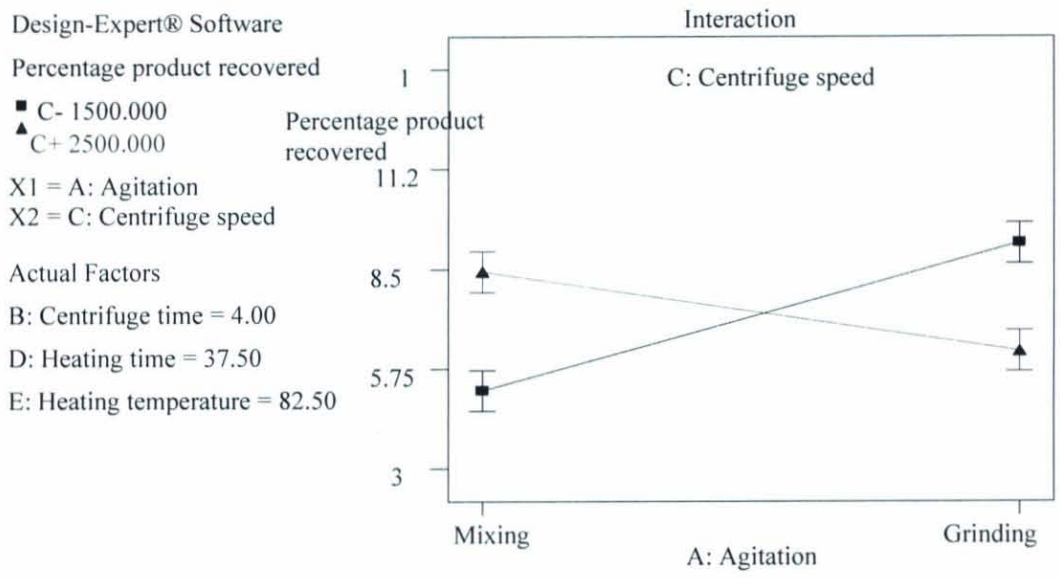


Figure B-4: Effect of the interaction between method of agitation and Centrifuge speed

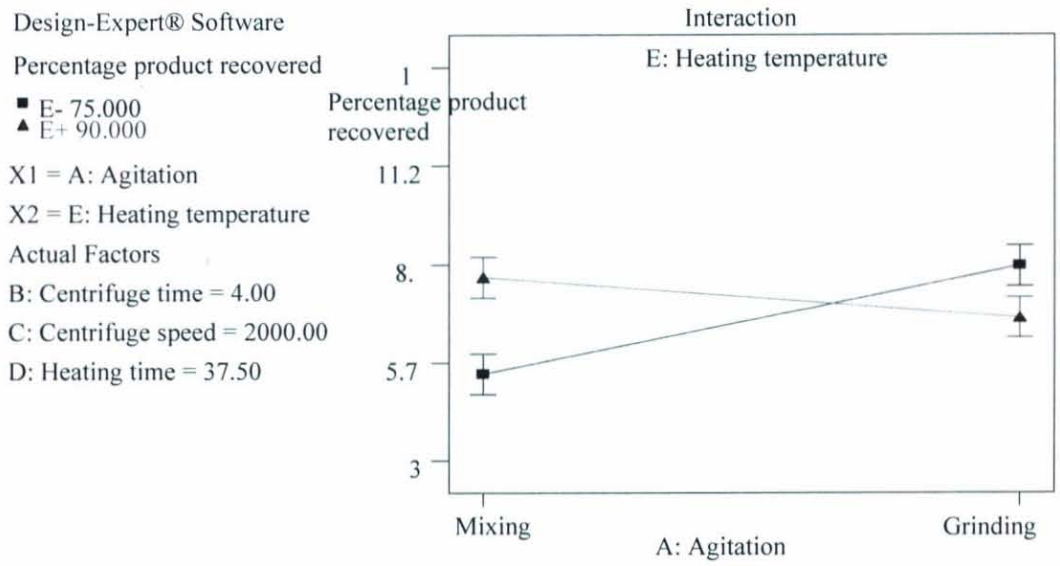


Figure B-5: Effect of the interaction between method of agitation and Heating temperature

Increasing the heating time from 30 to 45 min at the low heating temperature of 75 °C increased the percentage product recovered as indicated in Figure B-2, while at the high heating temperature (90 °C) the product decreased. Changing the method of agitation from mixing to grinding and heating at the high heating time of 45 min (Figure B-3) increased the product, but at the low of heating time (30min) the product recovery reduced. The interaction effect between method of agitation and centrifuge speed (Figure B-4) shows increasing agitation from mixing to grinding and centrifuging at the low speed (1500 x g) increased the product, while the opposite occurred at the high centrifuge speed. A similar effect resulted in increasing the agitation and heating temperature, where the highest product recovery was by, mixing and heating at 90 °C, or grinding and heating at 75 °C. The overall impact of the interaction effects on the product recovery is discussed in Chapter 5.

The impact from averages (centre points) of the above factors was investigated using an additional designed experiment and, the percentage product recovery results are given in Table B-4. The product recovery were higher than from the previous experiment (Table B-1)

Table B-4: Run combination and results for the center points of the experiment

Standard	Run	Block	Agitation	Centrifuge time (min)	Centrifuge speed (rpm)	Heating time (min)	Heating temperature (°C)	Percentage Product recovered (wt. %)
22	17	Block 2	Grinding	4	2000	37.5	82.5	18.44
21	18	Block 2	Mixing	4	2000	37.5	82.5	17.81
19	19	Block 2	Mixing	4	2000	37.5	82.5	14.93
24	20	Block 2	Grinding	4	2000	37.5	82.5	20.38
18	21	Block 2	Grinding	4	2000	37.5	82.5	11.56
25	22	Block 2	Mixing	4	2000	37.5	82.5	24.00
20	23	Block 2	Grinding	4	2000	37.5	82.5	15.80
26	24	Block 2	Grinding	4	2000	37.5	82.5	23.69
23	25	Block 2	Mixing	4	2000	37.5	82.5	20.51
17	26	Block 2	Mixing	4	2000	37.5	82.5	12.95

These results were combined with the results from the former experiment (Table B-1) to investigate overall impact to the product recovery at the average of factor levels (centre points). However, the two sets of results were analyzed as two blocks, due to the change of filter pore size (0.7 to 0.2 μm) and degradation of lipids over time. The percentage contribution of effects, Pareto chart and 1-way ANOVA, using Design-Expert indicated the effects that significantly impacted percentage product recovery, however the ANOVA assumptions were invalid. Therefore a natural log transformation of the percentage product recovery results was used for the statistical analysis, and the Pareto chart of the effect list is given in Figure B-6.

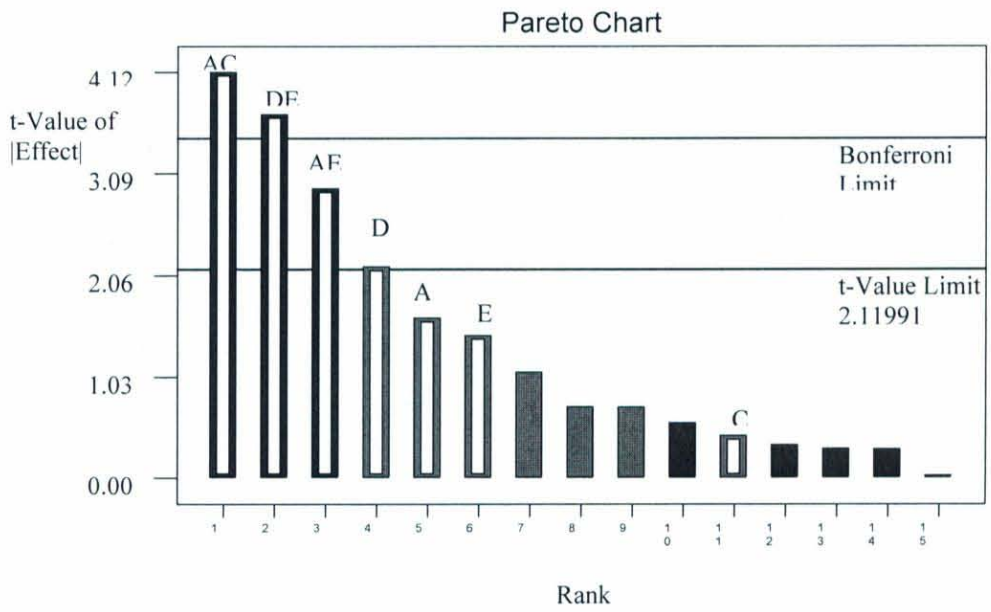


Figure B-6: Pareto chart of the factors and interaction effects of factors

The effects AC, DE, AE and D were the highest impact on product recovery. The statistical significance was evaluated using ANOVA results given in Table B-5. The model was significant in predicting the percentage recovery, however, the average of factor levels did not impact the product recovery as indicated by the insignificant curvature effect. The impact of carrying out the two experiments as two blocks was higher than any other individual/interaction effect, as indicated by the overall high mean square value.

Table B-5: 2-way ANOVA results for the center points of the experiment

Source	**Sum of squares**	**df**	**Mean square**	**F-value**	**p-value Prb>F**	
Block	5.53	1	5.53			
Model	1.96	7	0.28	6.81	0.0008	significant
A-Agitation	0.11	1	0.11	2.65	0.12	
C-Centrifuge speed	7.733×10^{-3}	1	7.733×10^{-3}	0.19	0.67	
D-Heating time	0.19	1	0.19	4.61	0.047	
E-Heating temperature	0.087	1	0.087	2.11	0.17	
AC	0.70	1	0.70	16.98	0.0008	
AE	0.35	1	0.35	8.62	0.01	
DE	0.56	1	0.56	13.63	0.002	
Curvature	0.046	1	0.046	1.13	0.30	Not significant
Residual	0.66	16	0.041			
Lack of Fit	0.12	8	0.015	0.22	0.98	Not significant
Pure Error	0.54	8	0.068			
Cor Total	8.19	25				

Although the impact of the averages of effects on product recovery was not significant, the results for centre points were all higher than the fractional points (Table B-1). Therefore, it is not entirely possible to conclude based on the above results, due to the significant impact of carrying the two sets of experiments in two different blocks.

Appendix C

Physical and Thermal Properties for FW1 and FW3

The viscosity results for product recovered from the cod and salmon whole fish mixed samples (FW1) at 25 °C and 40 °C are given in Tables C-1 and C-2.

Table C-1: Viscosity results for the FW1 product samples at 25 °C

Speed (rpm)		20	40	60	80	100	120	140	160	180	200	220	240
Shear Rate (s^{-1})		26.4	52.8	79.2	106	132	158	185	211	238	264	290	317
Sample	Vol. (mL)	Viscosity (cP)											
1	6.7	3.82	3.29	2.77	2.62	2.53	2.43	2.36	2.29	2.23	2.19	2.16	2.14
2	6.7	3.45	3.11	3.05	2.90	2.82	2.77	2.71	2.69	2.65	2.62	2.62	2.61
6	6.7	2.95	2.77	2.72	2.58	2.52	2.47	2.44	2.43	2.4	2.38	2.33	2.33
8	6.7	3.9	3.41	3.05	2.83	2.67	2.59	2.52	2.44	2.41	2.39	2.34	2.33
9	6.7	3.37	3.14	2.82	2.71	2.59	2.49	2.44	2.41	2.37	2.33	2.32	2.29
11	6.7	3.3	3.07	2.92	2.75	2.7	2.63	2.59	2.55	2.54	2.52	2.49	2.48
15	6.7	4.57	3.93	3.52	3.28	3.1	2.99	2.92	2.86	2.82	2.81	2.78	2.75
17	6.7	3.82	3.29	2.77	2.62	2.53	2.43	2.36	2.29	2.23	2.19	2.16	2.14
18	6.7	3.45	3.11	3.05	2.90	2.82	2.77	2.71	2.69	2.65	2.62	2.62	2.61
19	6.7	2.95	2.77	2.72	2.58	2.52	2.47	2.44	2.43	2.4	2.38	2.33	2.33
20	6.7	3.9	3.41	3.05	2.83	2.67	2.59	2.52	2.44	2.41	2.39	2.34	2.33
21	6.7	3.37	3.14	2.82	2.71	2.59	2.49	2.44	2.41	2.37	2.33	2.32	2.29
22	6.7	3.3	3.07	2.92	2.75	2.7	2.63	2.59	2.55	2.54	2.52	2.49	2.48
23	6.7	4.57	3.93	3.52	3.28	3.1	2.99	2.92	2.86	2.82	2.81	2.78	2.75
24	6.7	3.15	2.99	2.82	2.83	2.7	2.56	2.5	2.45	2.42	2.40	2.38	2.39
25	6.7	3.37	2.96	2.8	2.58	2.46	2.38	2.32	2.28	2.25	2.21	2.2	2.15

The specific heat capacity plots for the product recovered from FW1 are shown in the Figures C-1 and C-2. The plots are for samples; 1, 2, 3, 6, 8 and 9 and samples; respectively. The specific heat capacity values for the purified oil from both raw and frozen fresh salmon by products (FW3 – RS and FW3 – FS) are summarized in Table C-3

Table C-2: Viscosity results for the oil samples at 40 °C

Speed (rpm)		20	40	60	80	100	120	140	160	180	200	220	240
Shear Rate (s^{-1})		26.4	52.8	79.2	106	132	158	185	211	238	264	290	317
Sample	Vol. (mL)	Viscosity (cP)											
2	6.7	2.37	2.24	2.15	2.02	1.98	1.94	1.9	1.9	1.86	1.85	1.84	1.85
18	6.7	2.1	1.87	1.8	1.76	1.74	1.71	1.7	1.68	1.66	1.62	1.63	1.62
19	6.7	2.17	1.98	1.85	1.83	1.8	1.73	1.69	1.68	1.66	1.64	1.63	1.61
20	6.7	1.95	1.83	1.75	1.72	1.71	1.67	1.66	1.65	1.63	1.61	1.63	1.63
21	6.7	2.1	1.94	1.85	1.8	1.74	1.71	1.68	1.63	1.61	1.62	1.61	1.59
22	6.7	1.95	1.76	1.75	1.72	1.71	1.68	1.66	1.64	1.61	1.61	1.60	1.58
23	6.7	2.1	1.87	1.77	1.81	1.75	1.71	1.7	1.67	1.66	1.65	1.65	1.64
24	6.7	2.02	2.02	1.9	1.79	1.8	1.76	1.76	1.74	1.73	1.71	1.71	1.70
25	6.7	1.8	1.79	1.7	1.63	1.62	1.62	1.6	1.58	1.56	1.54	1.53	1.53

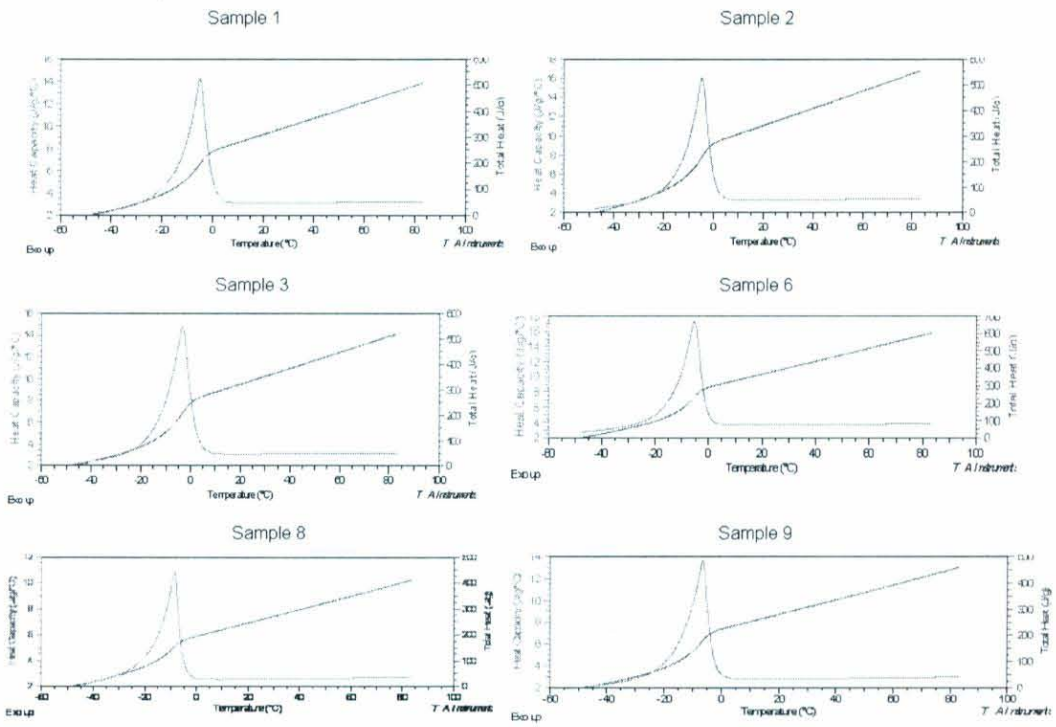


Figure C-1: Specific heat capacity plots for FW1 product samples 1, 2, 3, 6, 8 and 9

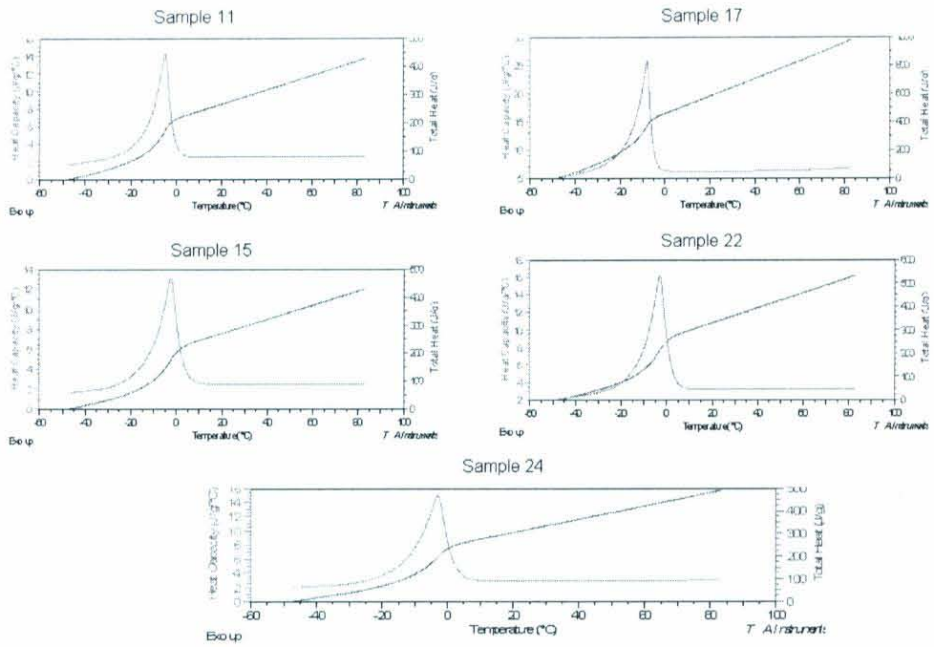


Figure C-2: Specific heat capacity plots for FW1 product samples 9, 11, 15, 17, 22 and 24

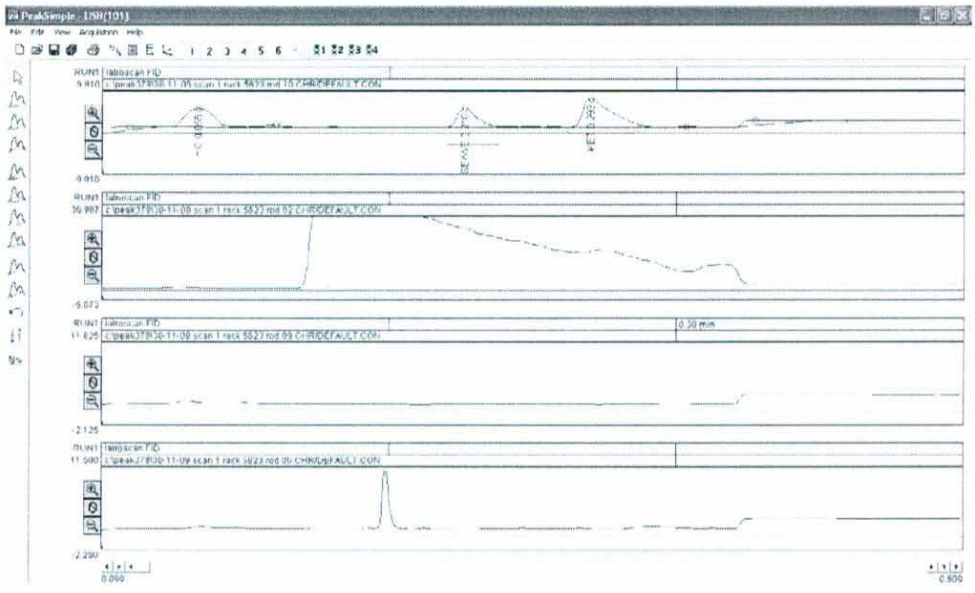
Table C-3: Specific heat capacity values for the purified oil between -50 °C and 140 °C

Temperature (°C)	R-1 (kJ/kg. °C)	R-2 (kJ/kg. °C)	R-3 (kJ/kg. °C)	F-1 (kJ/kg. °C)	F-2 (kJ/kg. °C)	F-3 (kJ/kg. °C)
-50	1.24 ± 0.12	1.66 ± 0.04	1.99 ± 0.30	1.30 ± 0.09	1.47 ± 0.37	1.41 ± 0.17
-40	1.40 ± 0.14	1.85 ± 0.04	2.27 ± 0.26	1.61 ± 0.04	1.81 ± 0.33	1.74 ± 0.13
-30	1.81 ± 0.31	2.31 ± 0.04	2.69 ± 0.19	2.04 ± 0.07	2.21 ± 0.18	2.14 ± 0.06
-20	2.97 ± 0.19	3.29 ± 0.16	3.85 ± 0.49	2.47 ± 0.06	2.86 ± 0.42	2.82 ± 0.22
-10	2.53 ± 0.20	3.30 ± 0.01	3.55 ± 0.19	3.00 ± 0.02	3.19 ± 0.31	3.11 ± 0.11
0	2.28 ± 0.21	2.88 ± 0.09	3.26 ± 0.44	2.89 ± 0.12	2.93 ± 0.30	3.00 ± 0.28
10	1.64 ± 0.19	2.03 ± 0.02	2.39 ± 0.25	1.84 ± 0.01	2.00 ± 0.26	1.96 ± 0.10
20	1.46 ± 0.18	1.83 ± 0.02	2.25 ± 0.35	1.57 ± 0.00	1.73 ± 0.26	1.72 ± 0.10
30	1.44 ± 0.19	1.81 ± 0.02	2.28 ± 0.43	1.52 ± 0.01	1.70 ± 0.27	1.67 ± 0.10
40	1.44 ± 0.19	1.81 ± 0.02	2.20 ± 0.29	1.52 ± 0.01	1.70 ± 0.26	1.67 ± 0.10
50	1.45 ± 0.19	1.82 ± 0.02	1.96 ± 0.15	1.52 ± 0.01	1.71 ± 0.26	1.67 ± 0.10
60	1.46 ± 0.19	1.82 ± 0.02	1.98 ± 0.14	1.52 ± 0.02	1.72 ± 0.26	1.67 ± 0.09
70	1.47 ± 0.19	1.83 ± 0.02	1.98 ± 0.14	1.52 ± 0.02	1.73 ± 0.26	1.68 ± 0.09
80	1.46 ± 0.19	1.83 ± 0.02	1.99 ± 0.14	1.53 ± 0.02	1.74 ± 0.26	1.68 ± 0.10
90	1.38 ± 0.13	1.85 ± 0.02	1.99 ± 0.14	1.52 ± 0.03	1.75 ± 0.26	1.68 ± 0.11
100	1.37 ± 0.12	1.85 ± 0.02	1.98 ± 0.14	1.52 ± 0.04	1.75 ± 0.26	1.68 ± 0.12
110	1.35 ± 0.11	1.84 ± 0.02	1.97 ± 0.13	1.51 ± 0.05	1.75 ± 0.26	1.67 ± 0.13
120	1.34 ± 0.11	1.85 ± 0.02	1.96 ± 0.13	1.50 ± 0.06	1.74 ± 0.27	1.66 ± 0.14
130	1.33 ± 0.11	1.84 ± 0.03	1.93 ± 0.12	1.49 ± 0.07	1.71 ± 0.31	1.63 ± 0.19
140	1.29 ± 0.09	1.86 ± 0.03	1.93 ± 0.13	1.45 ± 0.10	1.74 ± 0.30	1.63 ± 0.18

Appendix D

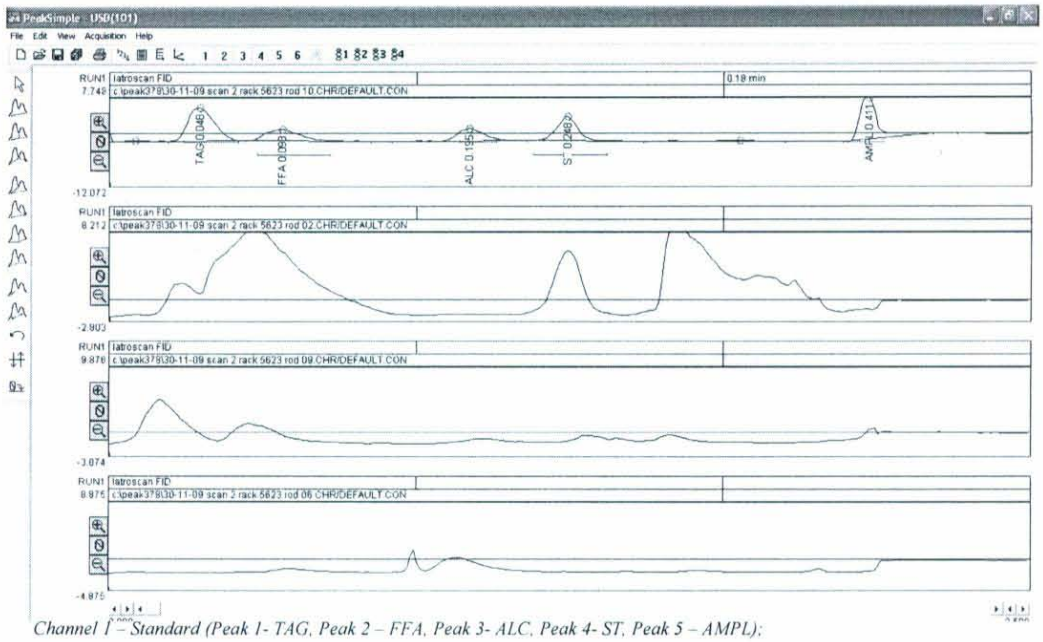
Iatroscan Peaks for the Waste and Recovered Product/Oil

Figures D-1, D-2 and D-3 present scans 1; hydrocarbons (HC), wax/steryl esters (WE/SE) and ketones (KET), scan 2; triacylglycerides (TAG), free fatty acids (FFA), alcohols (ALC) and sterols (ST) and scan 3; acetone mobile polar lipids (AMPL) and phospholipids (PL). The channel 1 (row 1) in all figures present the peaks for the standard and channels 2, 3 and 4 compare product and fish waste (FW1). The iatroscan peaks for lipids in FW2 - S waste and the product from FW1 are illustrated in Figures D-4 (HC, WE/SE, KET), D-5 (TAG, FFA, ALC, ST and part of AMPL) and D-6 (AMPL and PL). Results for the product from FW2 - S are given in Figures D-7, D-8 and D-9, and the peaks for the oil from FW3 – FS and FW3 - RS are illustrated in Figures D-10, D-11 and D-12. Again Channel 1 is for the standard.



Channel 1 – Standard (Peak 1- HC, Peak 2 – WE/SE, Peak 3- KET);
Channel 2 – FW1; Channel 3 – Sample 22 (block 2); Channel 4 – Sample 2 (block 1)

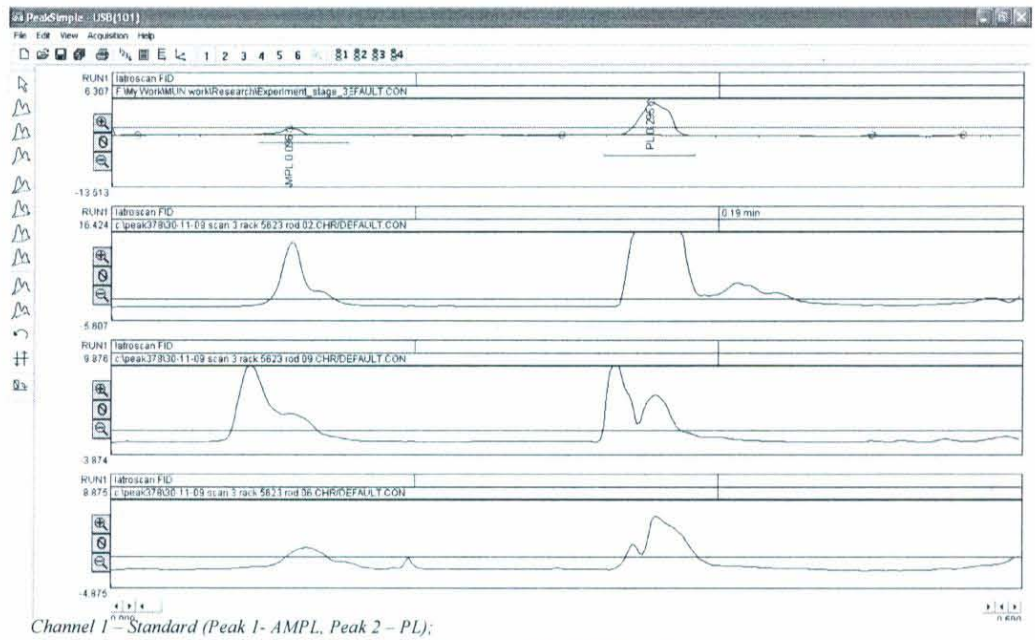
Figure D-1: Iatroscan 1 results for HC, WE/SE and KET in, product and waste of FW1



Channel 1 – Standard (Peak 1- TAG, Peak 2 – FFA, Peak 3- ALC, Peak 4- ST, Peak 5 – AMPL);

Channel 2 – FW1; Channel 3 – Sample 22 (block 2); Channel 4 – Sample 2 (block 1)

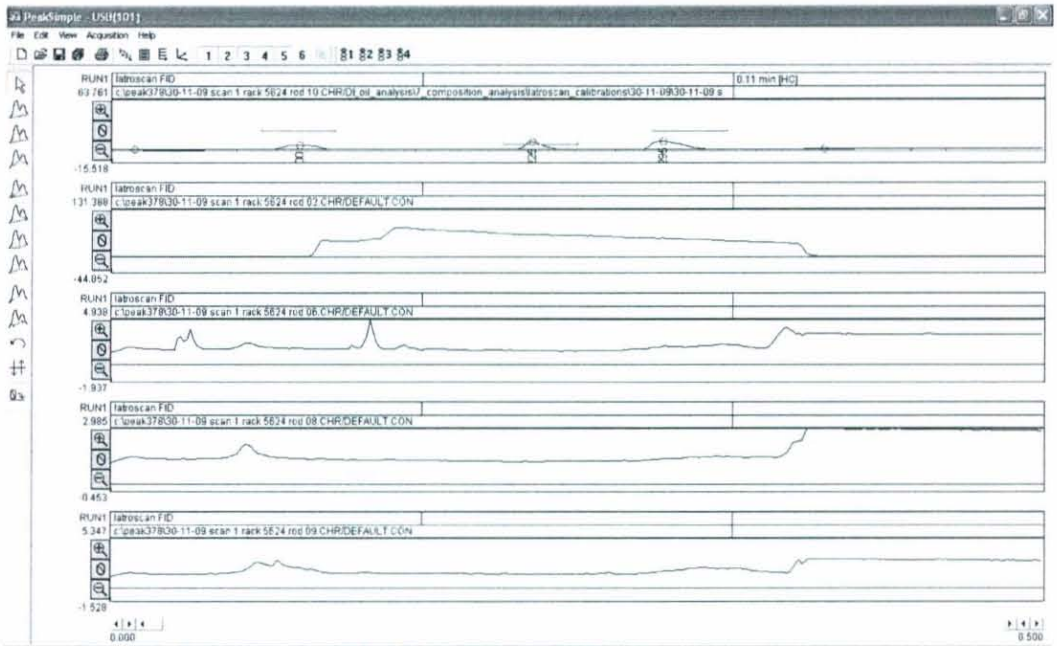
Figure D-2: Iatroscan 2 for TAG, FFA, ALC, ST and AMPL in product and waste of FW1



Channel 1 – Standard (Peak 1- AMPL, Peak 2 – PL);

Channel 2 – FW1; Channel 3 – Sample 22 (block 2); Channel 4 – Sample 2 (block 1)

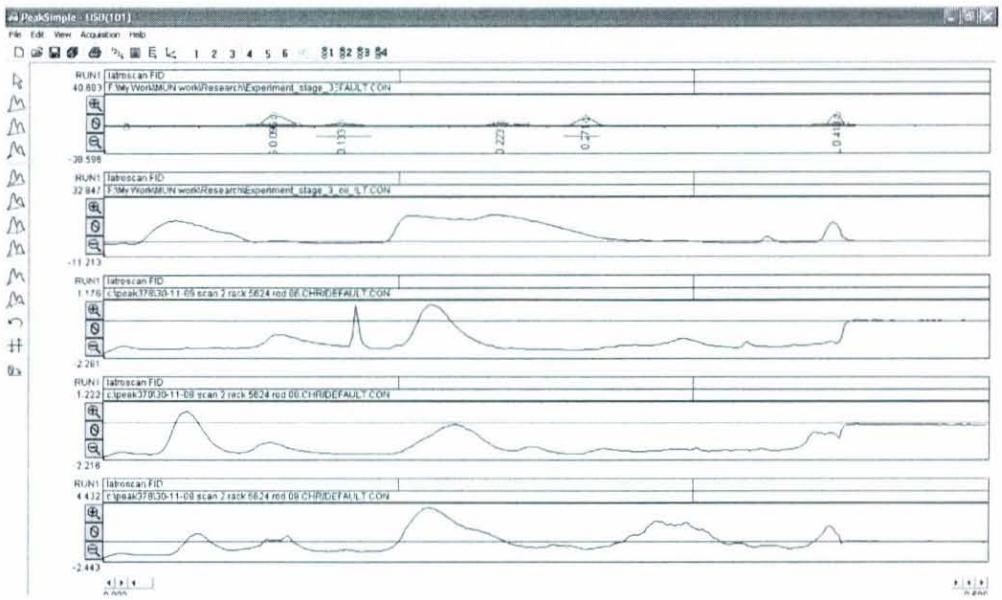
Figure D-3: Iatroscan 3 results for AMPL and PL in product and waste of FW1



Channel 1 – Standard (Peak 1- HC, Peak 2 – WE/SE, Peak 3- KET);

Channel 2 – FW2; Channel 3 – Sample 10; Channel 4 – Sample 23; Channel 5 – Sample 24

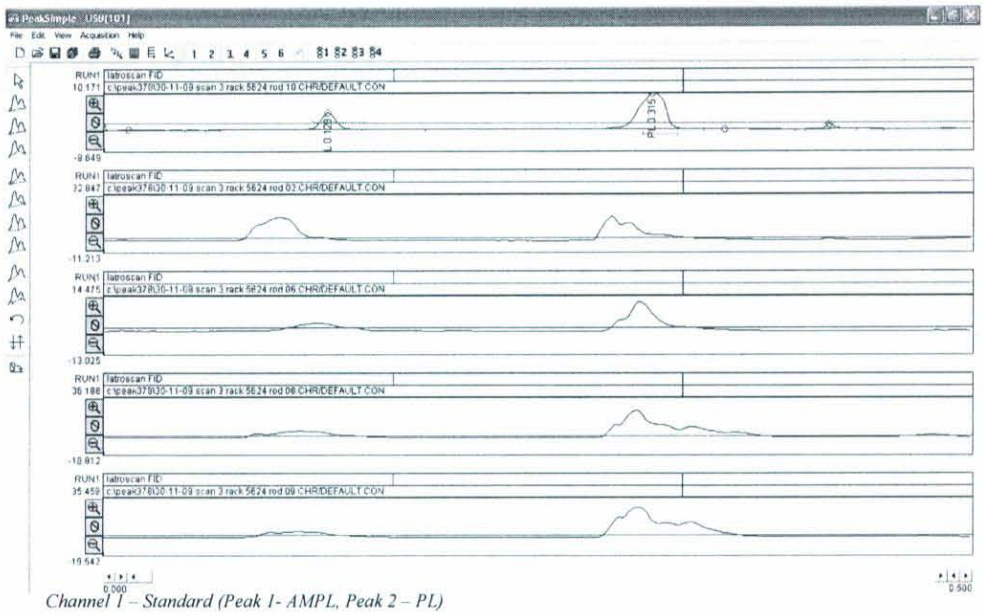
Figure D-4: Iatroscan peaks for HC, WE/SE and KET in FW2 - S waste and product of FW1



Channel 1 – Standard (Peak 1- TAG, Peak 2 – FFA, Peak 3- ALC, Peak 4- ST, Peak 5 – part of AMPL);

Channel 2 – FW2-S; Channel 3 – Sample 10; Channel 4 – Sample 23; Channel 5 – Sample 24

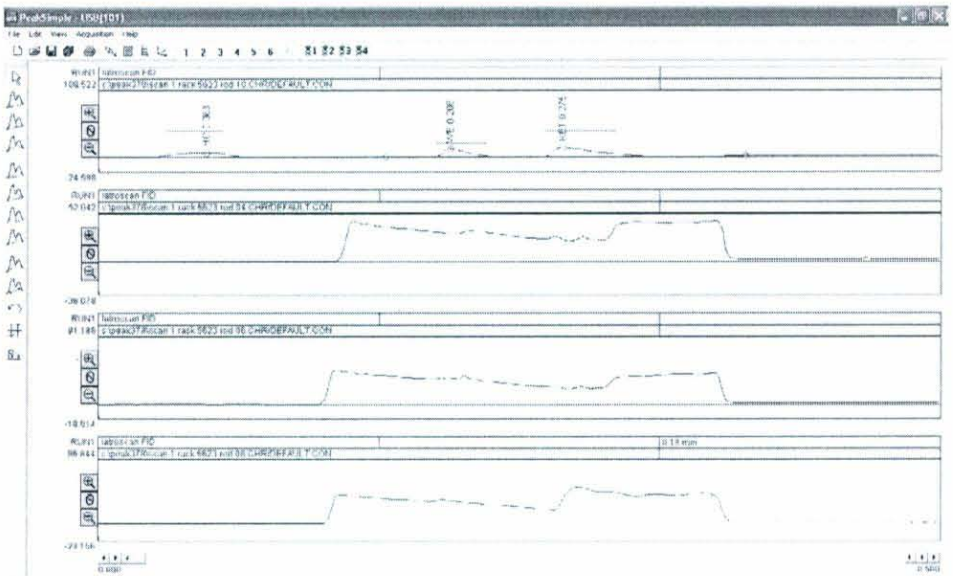
Figure D-5: Iatroscan peaks for TAG, FFA, ALC, ST and AMPL in, FW2 - S waste and the product from FW1



Channel 1 – Standard (Peak 1- AMPL, Peak 2 – PL)

Channel 2 – FW2; Channel 3 – Sample 10; Channel 4 – Sample 23; Channel 5 – Sample 24

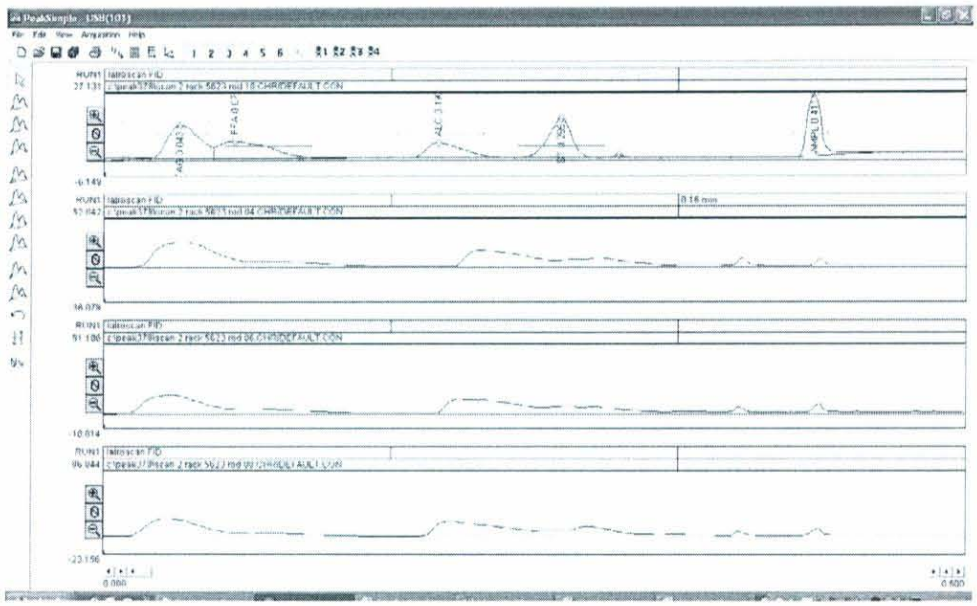
Figure D-6: Iatroscan peaks for AMPL and PL in FW2 - S waste and the product from FW1



Channel 1 – Standard (Peak 1- HC, Peak 2 – WE/SE, Peak 3 - KET)

Channel 2 – Ex1-1; Channel 3 – Sample Ex2-1; Channel 4 – Sample Ex3-1

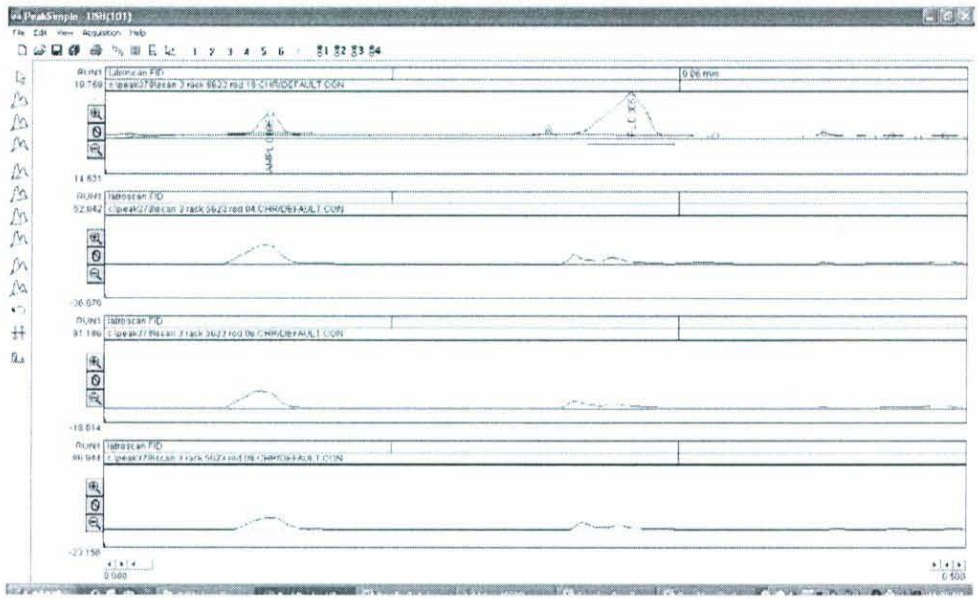
Figure D-7: Iatroscan peaks for HC, WE/SE and KET in the product from FW2 - S



Channel 1 – Standard (Peak 1- TAG, Peak 2 – FFA, Peak 3 – ALC, Peak 4 – ST, Peak 5 – AMPL)

Channel 2 – Ex1-1; Channel 3 – Sample Ex2-1; Channel 4 – Sample Ex3-1

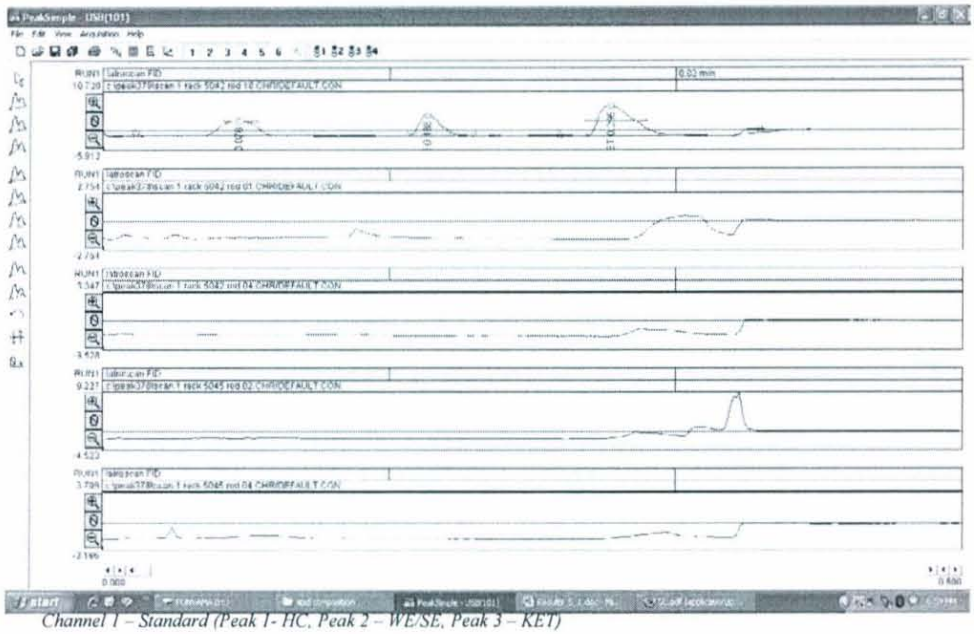
Figure D-8: Iatroscan peaks for TAG, FFA, ALC, ST and AMPL in the product from FW1



Channel 1 – Standard (Peak 1- AMPL, Peak 2 – PL)

Channel 2 – Ex1-1; Channel 3 – Sample Ex2-1; Channel 4 – Sample Ex3-1

Figure D-9: Iatroscan peaks for AMPL and PL in the product from FW2 - S

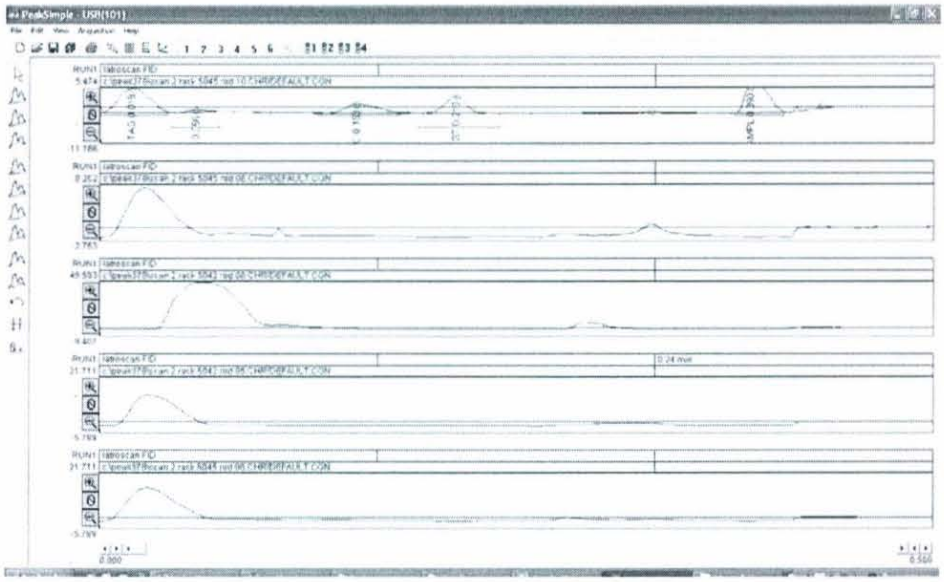


Channel 1 – Standard (Peak 1- HC, Peak 2 – WE/SE, Peak 3 – KET)

Channel 2 – Sample FW3-R1 (fish waste); Channel 3 – Sample FW3-R1 (purified oil);

Channel 4 – Sample FW3-F2 (fish waste); Channel 5 – Sample FW3-R1 (purified oil)

Figure D-10: Iatroscan peaks for HC, WE/SE and KET in the waste and purified oil from FW3

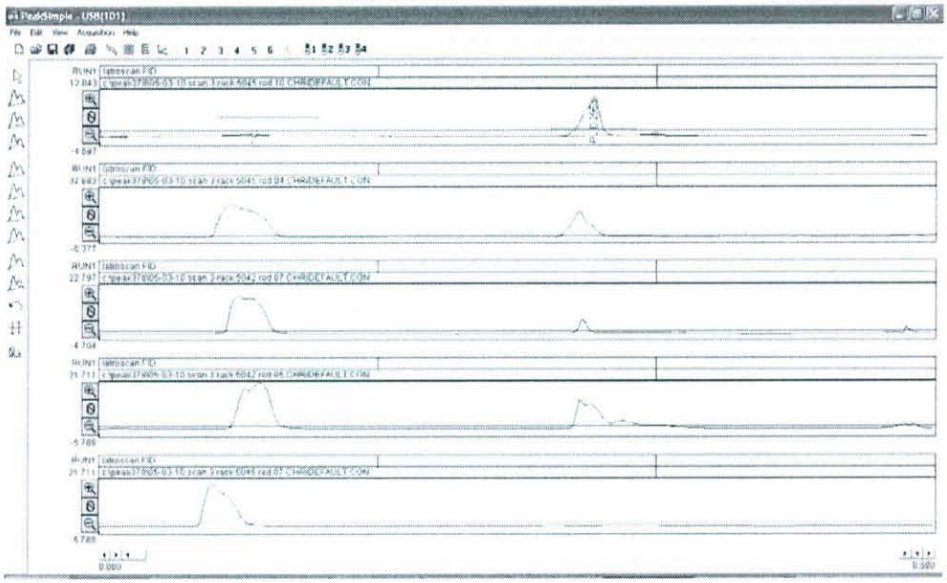


Channel 1 – Standard (Peak 1- HC, Peak 2 – FFA, Peak 3 – ALC, Peak 4 – ST, Peak 5 - AMPL)

Channel 2 – Sample FW3-R3 (fish waste); Channel 3 – Sample FW3-R2 (purified oil);

Channel 4 – Sample FW3-F2 (fish waste); Channel 5 – Sample FW3-F2 (purified oil)

Figure D-11: Iatroscan peaks for HC, WE/SE and KET in the waste and purified oil from FW3



Channel 1 – Standard (Peak 1- AMPL, Peak 2 – PL)

Channel 2 – Sample FW3-R1 (fish waste); Channel 3 – Sample FW3-R1 (purified oil);

Channel 4 – Sample FW3-F2 (fish waste); Channel 5 – Sample FW3-F1 (purified oil)

Figure D-12: Iatroscan peaks for AMPL and PL in, the waste and purified oil from FW3

References

1. AMEC (2003). Management of wastes from Atlantic seafood processing operations. AMEC Earth & Environmental Limited, 32 Troop Ave, Unit #301, Dartmouth, Nova Scotia

2. Arvanitoyannis I. and Kassaveti A. (2008). Fish industry waste: treatments, environmental impacts, current and potential uses. *International journal of food science and technology*, **43**, 726-745

3. CASD (2006). Inventory and Assessment of Marine sources of feedstock for Biodiesel in Newfoundland and Labrador. Center for Aquaculture and Seafood Development, Fisheries and Marine institute, Memorial University of Newfoundland, St. John's.

4. Carawan E. R. (1991). Processing plant waste management guidelines – Aquatic Fishery products. Department of food science, North Carolina State University. *Journal of Seafood & the environment – Pollution prevention short course*

5. Carawan E. R., Green D.P. and Frank B.T. (1986). Reduction in waste load from a seafood processing plant. Food science department and the North Carolina Agricultural Extension Service, North Carolina State University, Raleigh, NC 27605-7624

6. FRAZER RIVER (1994). Wastewater characterization of fish processing plant effluents. Frazer River Estuary management program, Frazer River Action plan, Environment Canada

7. FAO (1986). The production of fish meal and oil. Food and agriculture organization of the United Naitonas, Fisheries technical paper, 142, Rev. 1

8. Sathivel S., Prinyawiwatkul W. and Huang J. (2008). Thermal properties and applications of the Arrhenius equation for evaluating viscosity and oxidation rates of unrefined Pollock oil. Science Direct, *Journal of Food Engineering*, **84**, 187-193

9. Aidos I., Van der Padt A. Luten J. B. and Boom R. M. (2002b). Seasonal changes in crude and lipid composition of herring fillets, byproducts, and respective produced oils. J Agric Food chem., 50: 4589-99

10. Bechtel P.J. and Oliveira A. C. M. (2006). Chemical and liver lipid characterization of liver lipid and protein from cold-water fish species. *Journal of food science*, **71**, 6

11. Miyashita K., Takahashi R., Nishiyama T., Fukunaga K., Hosokawa M., Noguchi R. and Toyoshima K. (2004). Separation of Sardine oil without heating from Surimi waste and it's effect on lipid metabolism in rats. *Journal of Agricultural and food chemistry*, **52**, 2372-2375

12. Ivar S., Rustad T., and Slizyte R. (2005). Enzymatic hydrolysis of Cod (Gadus Morhua) by-products optimization of yield and properties of lipid and protein fractions. Science Direct, *Journal of process biochemistry*, **40**, 3680-3692

13. Wang J., Zhang F. and Preto F. (2008). A study on using fish oil as an alternative fuel for conventional combustors. CANMET Technology Centre, Natural Resources Canada. Science Direct, *Journal of fuel*, **87**, 2258-2268

14. Steigers J. A. (2003). Demonstrating the use of fish oil in a large stationary diesel engine. STEIGERS Corporation.

15. Balat M. (2008). Global trends on the processing of biofuels. *International journal of green energy*. **5**, 212-238

16. NREL (2004). Biomass oil analysis: Research needs and recommendations. National Renewable Energy Laboratory. Operated for the U.S. department of energy. Office of energy efficiency and renewable energy. Prepared by: Bozell J., Moens L., Petersen E., Tyson K. S. and Wallace R. Colorado

17. Balat M., Demirbas M. F. (2006). Recent advances on the production and utilization trends of biofuels: A global perspective. ScienceDirect. *Energy conservation & management*, **47**, 2371 – 2381

18. Faaij A. (2006). Modern biomass conversion technologies. Mitigation and adaptation strategies for global change 2006, ***11***, 343 – 375

19. Demirbas A., Pehlivan E. and Altun T. (2006). Potential evolution of Turkish agricultural residues as bio-gas, bio-char and bio-oil sources. *International journal of Hydrogen Energy*, **31**, 613-620

20. Rai A. K., Swapna H. C., Bhaskar N., Halami P.M. and Sachindra N.M. (2010). Effect of fermentation ensilaging on recovery of oil from fresh water fish viscera. Science Direct, *Journal of Enzyme and microbial technology*, **46**, 9-13

21. WISE (2004). Biodiesel in British Columbia – Feasibility study report. WISE Energy Co-opScience / Eco-Literacy Canada

22. Raheman H. and Ghadge S.V. (2006). Process optimization for biodiesel production from mahua (*Madhuca Indica*) oil using response surface methodology. Science Direct, *Journal of bioresource technology*, **97**, 379-384

23. Shonnard D. R., Marker T. and Kalnes T. (2007). Green Diesel: A Second Generation Biofuel. *International journal of chemical reactor engineering*, **5**, A48

24. Prasad R. and Srivastava A. (1999). Triglycerides-based diesel fuels. Science Direct, *Renewable and Sustainable Energy Reviews*, **4**, 111-133

25. Western Governors' Association (2008). Strategic assessment of bioenergy development in the west. Bioenergy Conversion Technology Characteristics. Final Report. Prepared by: Antares Group Inc.

26. Nelson R. G. and Schrock M. D. (2006). Energetic and economic feasibility associated with the production, processing, and conversion of beef tallow to a substitute diesel fuel. *Biomass and Bioenergy*, **30**, 584-591

27. Christie W. W. (2003). LIPID ANALYSIS: Isolation, Separation, Identification and Structural Analysis of Lipids. 3rd Edition, The Oily Press

28. Parrish C. C. (1998). Chapter 1: Determination of Total lipid, lipid classes, and fatty acids in aquatic samples Lipid in freshwater Ecosystems. Edited by: Arts M. T. and Wainman B. C. ISBN 0-387-98505-0 Springer-Verlag New York, Inc

29. Sanli H. and Canakci M. (2008). Biodiesel production from various feedstocks and their effects on the fuel properties. Springer, *Journal of industrial microbial biotechnology*, **35**, 431-441

30. Hanna M. A. and Ma F. (1999). Biodiesel production: a review[1]. Science Direct, *Journal of Bioresource Technology*, **70**, 1-15

31. http://www.nutritiondata.com/topics/fatty-acids Accessed 28/4/2010

32. Briggs T. E., Dunn R. O., Foglia T. A., Goering C. E., Marmer W. N. and Wu W. (1998). Low-temperature property and engine performance evaluation of ethyl and isopropyl esters of tallow and grease. *Journal of American Oil chemists' society*, **75**

33. Dunn R. O., Foglia T. A., Haas M. J., Hess M. A., Marmer W. N. and Wyatt V. T. (2005). Fuel property and nitrogen oxide emission levels of biodiesel production from animal fats. *Journal of American Oil chemists' society*, **82**, 585-591

34. Van de Voort F. R., Simpson B. K. and Aryee A.N.A. (2009). FTIR determination of free fatty acids in fish oils intended for biodiesel production. Science Direct, *Journal of process biochemistry*, **44**, 401-405

35. Skara T., Sivertsvik M. and Bickeland S. (2004). Production of Salmon oil from filleting by-products – effects of storage conditions on lipid oxidation and content of ω-3 Polyunsaturated fatty acids. *Journal of Food science*, **69**, 8, e417-e421

36. Sathivel S., Prinyawiwatkul W., Negulescu I. I. and King J.M. (2008). Determination of melting point, specific heat capacity and enthalpy of Catfish Visceral oil during the purification process *Journal of American oil chemist's society*, **85**, 291-296

37. Peterson C. L., Auld D. L. and Korus R. A. (1983). Winter rape oil fuel for diesel engines: Recovery and utilization. *Journal of American oil chemists' society*, **60**, 1579 – 1587

38. Goyal H. B., Saxena R. C. and Seal D. (2008). Bio-fuels from thermochemical conversion of renewable resources: A review, Science Direct. Renewable and Sustainable Energy Reviews, **12**, 504-517

39. Demirbas A. (2003). Biodiesel fuels from vegetable oils via catalytic and non-catalytic supercritical alcohol transesterifications and other methods: a survey. *Energy conservation and management*, **44**, 2093- 2109

40. IEA (2006). IEA Bioenergy. Annual report 2006. International Energy Agency

41. Chaala A. and Roy C. (2003). Recycling of meat and bone meal animal feed by vacuum pyrolysis. *Environmental Science and Technology*. **37**, 4517 – 4522

42. Adebanjo A. O., Dalai A. K. and Bakhshi N. N. (2005). Production of diesel like fuel and other value added chemicals from pyrolysis of animal fat, *Energy and Fuels*, **19**, 1735 – 1741

43. Johnson J., Brown N., Eason P. and Summer J. (1985). The nutritional quality of two types of fish silage for boiler chickens. *Journal of Sci Food Agric*, **36**, 1051-1056

44. Garcia-Sanda E., Omil F. and Lema J. M. (2003). Clean production in fish canning industries: recovery and reuse of selected wastes. *Journal of Clean Technology Environmental Policy*, **5**, 289-294

45. Seafish technology (2001). Fish waste production in the United Kingdom - The quantities produced and opportunities for better utilization. Sea fish Industry Authority

46. Gopalakrishna A. G., Kimura T., Nandini K. E., Nakajima M., Raghavarao K. S. M. S., Sheila P. M. and Subramanian R. (2000). Membrane processing of used frying oils. *Journal of American Oil Chemists' Society*, **77**, 3, 323-328

47. Kawakatsu T., Nakajima M. and Subramanian R. (1999). Processing of vegetable oils using polymeric composite membranes. ScienceDirect. *Journal of food Engineering*, **38**, 1, 41-56

48. Kimura T., Raghavarao K. S. M. S., Nakajima M. and Subramanian R. (2004). Processing vegetable oils using non denser polymeric composite membranes. *Journal of American Oil Chemists' Society*, **81**, 4, 313-322

49. Nakajima M. and Snape J. B. (1996). Processing of agricultural fats and oils using membrane technology. *Journal of food Engineering*, **30**, 1-2, 1-41

50. Manjula S. and Subramanian R. (2006). Membrane technology in degumming, dewaxing, deacidifying, and decolorizing edible oils. *Critical reviews in Food Science and Nutrition*, **46**, 569 – 592.

51. https://www.vwrcanlab.com/catalog/product/index.cgi?catalog_number=28497-973&inE=1&highlight=28497-973 Accessed 20/1/2010

52. Chayamizu H. and Kikuchi H. (1988). Dewaxing of sunflower oil by microza TP-113. In proceedings of; Session lectures and scientific presentation on ISF-JOCS World Congress, I, 1988

53. Iwama A. (1987). New process for purifying soybean oil by membrane separation and economical evaluation of the process. *Journal of American Oil Chemists' society*, **64**, 1258.

54. Pyrde E. H. (1983). Vegetable oil as diesel fuel: Overview. *Journal of American oil chemists' society*, **60**, 1557 – 1558

55. Naik S. N., Vidya Sagar D. and Meher L.C. (2006). Technical aspects of biodiesel production by transesterification – a review. Science Direct, *Renewable and Sustainable Energy Reviews*, **10**, 248-268

56. Mittelbach M. and Schober S. (2007). Iodine value and biodiesel: Is limitation till appropriate?. WILEY Interscience, *Journal of lipid technology*, **19**, 12

57. Anon (1982). Filtered used frying fat powers diesel fleet. *Journal of American oil chemists' society*, **59**, 780A – 781A

58. Harwood H. J. (1984). Oleochemicals as a fuel: Mechanical and economic feasibility. *Journal of American oil chemists' society*, **61**, 315 – 324

59. Ma F. and Hanna M. A. (1999). Biodiesel production: a review. *Bioresource Technology*, **70**, 1-15

60. Ghadge S. V. and Raheman H. (2005). Biodiesel production from mahua (*Madhuca Indica*) oil having high free fatty acids. *Biomass and energy*, 28, 6, 601-605

61. Canakci M. and Sanli J. (2008). Effect of different alcohol and catalyst usage on biodiesel production from different vegetable oils. *Energy & Fuels*, **22**, 2713-2719

62. Ma F., Clements L. D. and Hanna M. A. (1999). The effect of mixing on a transesterification of beef tallow. Bioresource Technology, 69, 289- 293

63. Bradshaw G. B. and Meuly W. C. (1944). Preparation of detergents. US Patent 2, 360-844

64. Tanaka Y., Okabe A. and Ando S. (1981). Method for the preparation of lower alkyl esters of fatty acids. US Patent 4, 303- 590

65. Ali Y. (1995). Beef tallow as a biodiesel fuel. PhD dissertation. Biological Systems Engineering, University of Nebraska- Lincoln

66. Zhang D. (1994). Crystallization characteristics and fuel properties of tallow methyl esters. Master thesis, *Food science and technology*, University of Nebraska- Lincoln

67. Freedman B., Pryde E. H. and Mounts T. L. (1984). Variables affecting the yields of fatty esters from transesterified vegetable oils. *Journal of American oil chemists' society*, **61**, 1638-1643

68. Pegg M. J., and Joshi R.M. and (2007). Flow properties of biodiesel fuel blends at low temperatures. Science Direct, *Journal of Fuel*, **86**, 143-151

69. El-Mashad H. M. and Zhang R. (2007). Bio-diesel and bio-gas production from seafood processing by-products. *Maximizing the value of marine by-products*, Wood head publishing in food science, technology and nutrition, **22**, 460-485

70. Hartman L. (1956). Methanolysis of triglycerides. *Journal of American oil chemists' society*, **61**, 315-324

71. Ma F., Clements L. D. and Hanna M. A. (1998a). The effects of catalyst, free fatty acids and water on transesterification of beef tallow. *Trans. ASAE 41*, 1261-1264

72. Ahn E., Mittelbach M. and Marr R. (1995). A low waste process for the production of biodiesel. *Sep Sci Technol*, 39, 7-9, 2021- 2033

73. Ma F., Clements L. D. and Hanna M. A. (1998a). The effects of catalyst, free fatty acids and water on transesterification of beef tallow. *Trans. ASAE 41*, 1261-1264

74. Ahn E., Mittelbach M. and Marr R. (1995). A low waste process for the production of biodiesel. *Sep Sci Technol*, 39, 7-9, 2021- 2033

75. Wright H. J., Segur J. B., Clark, H. V., Coburn S. K., Langdon E. E. and Dupuis R. N. (1944). A report on ester interchange. *Oil and soap*, 21, 145 – 148

76. Turck R. (2002). Method for producing fatty acid esters of monovalent alkyl alcohols and use thereof. USP 0156305.

77. Keim G. I. (1945). Process for treatment of fatty glycerides. US Patent 2, 383 – 601

78. Jackson M. A. and King J. W. (1996). Methanolysis of Triglycerides. *Journal of American oil chemists' society*, **33**, 129-132

79. Linko Y-Y, Lamsa M., Huhtala A. and Linko P. (1994). Lipase-catalyzed transesterification of rapeseed oil and 2-ethyl-1-hexanol. *Journal of American oil chemists' society*, **71**, 1411-1444

80. Mittelbach M. (1990). Lipase catalyzed alcoholysis of sunflower oil. *Journal of American oil chemists' society*, **67**, 3, 168-170

81. Cao W., Han H. and Zhang J. (2005). Preparation of biodiesel from soybean oil using supercritical methanol and co-solvent. *Fuel*, **84**, 347- 351

82. Madras G., Kolluru C. and Kumar R. (2004). Synthesis of biodiesel in supercritical fluids. *Fuel* , 83, 2029- 2033

83. Smith M. K. (1949). Process of producing esters. US Patent 2, 444- 486

84. Lozano P., Chirat N., Graille J. and Pioch D. (1996). Measurement of free glycerol in biofuels. Fresenius. *J Anal Chem*, **354**, 319–22.

85. Knothe G. (2001). Analytical methods used in the production and fuel quality assessment of biodiesel. *Trans ASAE*, **44**, 2, 193–200.

86. Holcapek M., Jandera P., Fischer J. and Prokes B. (1999). Analytical monitoring of the production of biofuel by high performance liquid chromatography with various detection methods. *J Chromatogr A*, **858**, 13–31

87. Noureddini H. and Zhu D. (1997). Kinetics of transesterification of soybean oil. *J Am Oil Chem Soc*, **74**, 11, 1457–63.

88. Darnoko D., Cheryan M. and Perkins E.G. (2000). Analysis of vegetable oil transesterification products by gel permeation chromatography. *J Liq Chrom Rel Technol*, **23**, 15, 2327 – 2335

89. Meier H. F., Barros A. A. C., Madureira L. A. S., Wisniewski jr A. and Wiggers V. R. (2009). Biofuels from waste fish oil pyrolysis: Continuous production in a pilot plant. Science Direct, *Journal of fuel*, **88**, 2135-2141

90. Grossley T. D., Heyes T. D. and Hudson B. J. F. (1962). The effect of heat on pure triglycerides. *Journal of American oil chemists' society*, **39**, 9-14

91. http://www.simscience.org/membranes/advanced/essay/surfactants.html Accessed 28/4/2010

92. Murakami S., Kojima T., Kunisawa K. and Shigeru K. (2004). Evaluation of ozone treated fish waste oil s a fuel for transportation. *Journal of Chemical Engineering of Japan*, **37**, 863-870

93. Mittelbach M., Pokits B. and Silberholz A. (1992). Production and fuel properties of fatty acid methyl esters from used frying oil. In: Liquid Fuels from Renewable Resources: Proceedings of an Alternative Energy Conference, St. Joseph, Mich. *ASAE*, 74–78

94. Environmental Benefits. National Biodiesel board, www.biodiesel.org/pdf_files/fuelfactsheets/Enviro_Benefits.PDF

95. Lee I., Johnson L. A. and Hammond E. G. (1996). Reducing the crystallization temperature of biodiesel by winterizing methyl soyate. *Journal of American oil chemists' society*, **73**, 5, 631-636

96. Dunn R. O., Shockley M. W. and Bagby M. O. (1996). Improving the low temperature properties of alternative diesel fuels: vegetable oil-derived methyl esters. *Journal of American oil chemists' society*, **73**, 10, 1719-1728

97. Natural Resources Canada (2004). An evaluation of marine based biodiesel using GHGenius. Office of energy efficiency. Ottawa, Ontario

98. Goering C. E., Camppion R. N., Schwab A. W. and Pryde E. H. (1982b). In vegetable oil fuels, proceedings of the international conference on plant and vegetable oils as fuels, Fargo, North Dakota. American Society of Agricultural Engineers, St. Joseph, MI 4, 279-286

99. Ziejewski M., Kaufman K. R., Schwab A. W. and Pryde E. H. (1984). Diesel engine evaluation of a nonionic sunflower oil-aqueous ethanol microemulsion. *Journal of American oil chemists' society*, **61**, 1620 – 1626

100. Goering C. E. and Fry B. (1984a). Engine durability screening test of a diesel oil/ soy oil/ alcohol microemulsion fuel. *Journal of American oil chemists' society*, **61**, 1627 – 1632

101. Goering C. E. (1984b). Final report for project on effect of nonpetroleum fuels on durability of direct-injection diesel engines under contract. 59-2171-1-6-057-0, USDA, ARS, Peoria, IL

102. USEPA (2002). A comprehensive analysis of biodiesel impacts on exhaust emissions. Draft Technical Report. Assessment and Standards Division Office of Transportation and Air Quality. United States Environmental Protection Agency. EPA420-P-02-001

103. Natural Resources Canada (2002). Assessment of biodiesel and ethanol diesel blends, greenhouse gas emissions, exhaust emissions, and policy issues. Prepared for: Natural Resources Canada, by Levelton Engineering Ltd. in association with, $(S\&T)^2$ Consultants Inc.

104. Sathivel S., Prinyawiwatkul W., Grimm C.C., King J.M. and Lloyd S. (2002). Fatty acid composition of crude oil recovered from Catfish Viscera. *Journal of American Oil chemists's society*, **79**, 10

105. Omil F., Mendez R. and Lema J. M. (1995). Anaerobic treatment of saline wastewaters under high sulphide and ammonia content. *Bioresource Technology*, **54**, 269-278

106. Carawan R. E., Chambers J. V., Zalli R. R. and Wilkowske (1979). Water and wastewater management in food processing. Spin-off on seafood water and wastewater management. Extension special report No. AM-18F, January, North Carolina State University, Cornell University and Purdue University, Raleigh, North Carolina, USA

107. El-Dars M. S., Fahim A. F., Fleita D. H. and Ibrahim A. M. (2000). Evaluation of some methods for fish canning wastewater treatment. Department of Biochemistry, Faculty of Science, Helwan University, Egypt.

108. Terashima M., Takahashi Y., and Yoshida H. (2003). A simplified reaction model for production of oil, Amino acids, and organic acids from fish meat by hydrolysis under sub-critical and supercritical conditions *Journal of Chemical Engineering of Japan*, **36**, 441-448

109. Lillford P.J., Edwards M. F. and Wagner A. (2008). Clean technology in food processing. *Journal of Philosophical transactions: Mathematical, Physical and Engineering Sciences*, **335**, 1728, 1363-1371

110. Babbitt J. K. (1990). Intrinsic quality and species of North Pacific fish. In Proceedings of International By-products Conference, April 25-27, Alaska Sea Grant College Program, University of Alaska Fairbanks, Alaska, USA

111. Sun T., Prinyawiwatkul W. and Zhimin X. (2006). FA Composition of oil extracted from farmed Atlantic Salmon Viscera. *Journal of American oil chemist's society*, **83**, 291-296

112. Ackerman R. G. (1995). Seafood Lipids. Seafoods: Chemistry, Processing Technology and Quality, edited by F. Shahidi and J. R. Botta, Kluwer Academic/ Plenum, New York, pp. 35 – 48

113. Terashima M., Takahashi Y., and Yoshida H. (2003). A simplified reaction model for production of oil, Amino acids, and organic acids from fish meat by hydrolysis under sub-critical and supercritical conditions *Journal of Chemical Engineering of Japan*, **36**, 441-448

114. Lillford P.J., Edwards M. F. and Wagner A. (2008). Clean technology in food processing. *Journal of Philosophical transactions: Mathematical, Physical and Engineering Sciences*, **335**, 1728, 1363-1371

115. Sathivel S., Prinyawiwatkul W., Grimm C. C., King J. M. and Lloyd S. (2002) FA composition of crude oil recovered from catfish viscera. *Journal of American Oil Chemists' Society*, **79**, 989–992

116. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC543702/pdf/jbacter00786-0078.pdf Accessed 1/09/2010

117. Shahidi F. and Kamil J. Y. V. A. (2002). Enzymes from fish and aquatic invertebrates and their application in the food industry. *Trends Food Sci Technol*, **13**, 1-30

118. Mohr V. (1977). Fish protein concentrate production by enzymic hydrolysis. In: Adler-Nissen, et. al., editors. Proceedings of the symposium A3 on biochemical aspects of new protein food, FEBS Federation of European biochemical societies 11th meeting, **44**, 53-62

119. Shahidi F., Han X-Q and Synowiecki, J. (1995). Production and characteristics of protein hydrolysates from capelin (Mallotus villosus). Food Chem, 53, 285- 293

120. Liaset B., Julshamn K. and Espe M. (2003). Chemical composition and theoretical evaluation of the produced fractions from enzymic hydrolysis of salmon frames with Protamex^{TM}. *Process Biochem*, **38**, 1747 – 1759

121. Slizyte R., Dauksas E., Falch E., Storro I. and Rustad T. (2005). Yield and composition of different fractions obtained after enzymatic hydrolysis of cod (Gadus Morhua) by-products. *Process Biochem*, **40**, 1415 – 1425

122. Kazunobu T., Sachiko S. and Wataru K. (1996). Manufacture of protein hydrolyzates with protease and lipid-degrading enzymes. Patent JP 08308507

123. Yoshida H., Terashima M. and Takahashi Y. (1999). Production of Organic Acids and Amino Acids from Waste Fish meat by Subcritical Water Hydrolysis, *Biotechnol. Progress*, 15, 1090-1094

124. Ahmed J. and Mahendrakar N. S. (1995). Effect of different levels of molasses and salt on acid production and volume of fermenting mass during ensilaging of tropical fresh water fish viscera. *Journal of Sci Food Agric*, **32**, 115-118

125. Bhaskar N., Suresh P. V., Sakhare P. Z. and Sachindra N. M. (2006). Shrimp bio waste fermentation with Pediococcus acidolactici CFR2182: optimization of fermentation conditions by response surface methodology and effect of optimized conditions on deproteination/demineralization and carotenoid recovery. *Enzyme Microb Technology*, **40**, 1427-1434

126. Zakaria Z., Hall G. M. and Shama G. (1998). Lactic acid fermentation of scampi waste in a rotating horizontal bioreactor for chitin recovery. *Process Biochem*, **33**, 1–6.

127. Schroder K., Clausen E., Sandberg A. M. and Raa J. (1980) Psychrotrophic Lactocacillus plantarum from fish and its ability to produce antimicrobial substance. In: ConnellJJ, editor. Advances in fish science and technology. *Farnham, Surrey, England: Fishing News Books*, 480–512.

128. Raa J. and Gildberg A. (1982). Fish silage: a review. *Crit Rev Food Sci Nutr*, **16**, 383–419.

129. Piccolo T. (2003). Framework analysis of fish waste to biodiesel production – Aquafinca – case study. Aquatic Biofuels – Tony Piccolo. Energy and Sustainable Development.

130. Godiganur S., Murthy C. S. and Reddy R.P. (2010). Performance and emission characteristics of a Kirloskar HA394 diesel engine operated on fish oil methyl esters. Science Direct, *Journal of Renewable Energy*, **35**, 335-359

131. Lin C. and Li R. (2009). Fuel properties of biodiesel produced from the crude fish oil from the soapstock of marine fish. Science Direct, *Journal of fuel processing technology*, **90**, 130-136

132. Tan, C. P. & Che Man, Y. B. (2002). Differential scanning calorimetric analysis of palm oil, palm oil based products and coconut oil: Effect of scanning rate variation. *Food Chemistry*, **76**, 89–102.

133. Sathivel S. (2005). Thermal and flow properties of oils from salmon head. *Journal of American oil chemists' society*, 81, 147-151

134. Hassel R. L. (1976). Thermal analysis: An alternative method of measuring oil stability. *Journal of the American Oil Chemists' Society*, **53**, 79–181

135. Zhang H-Z. and Lee T-C. (1997). Rapid near-infrared spectroscopic method for the determination of free fatty acid in fish and its application in fish quality assessment. *J Agric Food Chem*, **45**, 3515–3521.

136. Isaksson T., Tøgersen G., Iversen A. and Hildrum K. I. (1995). Non-destructive determination of fat, moisture and protein in salmon fillets by use of near-infrared diffuse spectroscopy. J *Agric Food Chem*, **69**, 95–100.

137. Frankel E. N. (1998). Methods to determine extent of oxidation. In E. N. Frankel (Ed.). Glasgow, UK: The Oil Press. *Lipid oxidation*, 79–98

138. Takeuchi M., Hara S., Totani Y., Hibino H. and Tanaka Y. (1997). Autoxidative behaviour of polyunsaturated phospholipids. I. Oxidative stability of marine oil containing polyunsaturated phospholipids. Journal of Jpn Oil Chemists's society, 46, 175 – 181

139. Young F. V. K. (1986). The chemical and physical properties of crude fish oils for refiners and hydrogenators. In Fish Oil Bulletin No. 18. Herefordshire, United Kingdom: Fish oil International Association of Fish Meal Manufactures

140. Blythe N. X. (1996). Fish oil as an alternative fuel for internal combustion engines. Fairbanks Morse Engine Division. Coltec Industries Inc. ICE-Vol 26-3, 1996 Spring Technical Conference. *ASME 1996*, **3**

141. Ocean Nutrition Canada (2004). Fish to fuel, Waste to resource, A 2004 Eco-Efficiency Success story. Ocean Nutrition Canada, Mulgrave, Nova Scotia

142. Steigers J. (2005). Alaska fish oil as a biodiesel feed stock. STEIGERS Corporation. Presentation on: The challenging face of energy in rural Alaska 2005, Valdez, Alaska. September 21, 2005, *Journal of Renewable Energy*, **35**, 335-359

143. Dip-Ing Jose N Carrinco, (February 17, 2005). MAN B&W do Brasil. Green power from diesel engines burning biological oils and recycled fat. Paper presented at the Rio5-world climate & energy international congress, Rio de Joneiro, Brazil

144. Lin C. and Li R. (2009). Engine performance and emission characteristics of a marine fish-oil biodiesel produced from the discarded parts of marine fish. Science Direct, *Journal of fuel processing technology*, **90**, 883-889

145. Lanari D. and Franci C. (1998). Biogas production from solid wastes removed from fish farm effluents. *Aquatic Living Resources*, **11**, 289–295.

146. Solbakken J., Hesjevik J.I., Liltved H., Johannson R., Windmar L. and Vogelsang C. (2005). Beste tilgjengelige teknikker forfiskeoppdretti Norden. In: TemaNord, 5–139. (in Norwegian)

147. Folkecenter (2005). Folkecenter biogas technology for licence production. http://www.folkecenter.dk/en/biogas/Folkecenter_biogas_technology pdf.

148. Hartman K., Zhang R., Antunes M., Chornet E., Turnbull J. and Shoemaker S. (2001). Anaerobic digestion of fish and wood wastes for biogas production. ASAE paper No 01-6014

149. Feijoo G., Soto M., Mendez R. and Lema I. M. (1995). Sodium inhibition in the anaerobic digestion process. Antagonism and adaptation phenomena. *Enzyme and Microbial Technology*, **17**, 180-188

150. Rodenhizer J. S. and Boardman G. D. (1999). Collection, analysis, and utilization of biogas from anaerobic treatment of crab processing waters. *Journal of aquatic Food product Technology*, **8**, 2, 59-67

151. O'Keefe D. M., Owens J. M. and Chynoweth D. P. (1996). Anaerobic composting of crab-picking wastes for byproduct recovery. *Bioresource Technology*, **58**, 265-272

152. Callagan F. I., Wase D. A. I., Thayanithy K. and Forster C. F. (1999). Co-digestion of waste organic solids: batch studies. *Bioresource Technology*, **67**, 117-122

153. Palenzuela-Rollon A. (1999). Anaerobic processing of fish processing waste water with special emphasis on hydrolysis of suspended solids. PhD thesis, Wageningen University, Wageningen, The Netherlands

154. Achour M., Khelim O., Bouazizi I. and Hamdi M. (2000). Design of an integrated bioprocess for the treatment of tuna processing liquid effluents. *Process Biochemistry*, **35**, 1013-1017

155. Vidal G., Aspe E., Christina Marti M. and Roeckei M. (1997). Treatment of recycled wastewaters from fishmeal factory by an anaerobic filter. *Biotechnology Letters*, **19**, 2, 117-121

156. Araya Ken et. al. (1987). Diesel engine performance with sunflower oil and fish oil. *J Senshu University Hokkaido (Nat Sci)*, 20, 137–54.

157. BROOKFIELD. A guide to getting more from your Brookfield viscometer. More solutions to sticky problems. Brookfield Engineering Laboratories, Inc. Middleboro, USA.

158. Standard Methods for the Examination of Water and Wastewater (1995), 19th edition

159. Sawyer and McCarthy (1978). Chemistry for Environmental Engineers, 3rd Edition, New York: McGraw-Hill, PP 454-463

160. STANDARD METHODS: for the examination of water and wastewater (1971), 13th Edition, American Public Health Association, 1015 Eighteenth Street, N.W. Washington, D.C. 20036, pgs 110-112 and pgs 489-495

161. SRI Inc. PeakSimple. Copyright 1994 – 2000 Alan Hetherington. Version 3.78 (6 channel)

162. Stat-Ease Inc. Design Expert. Minneapolis, MS, USA, Version 7.1.3 Trial

163. Manual No. M/98-211-B0104. Operating instructions, BROOKFIELD DV-III ULTRA programmable Rheometer. BROOKFIELD Engineering laboratories Inc. Middleboro, USA

164. BROOKFIELD. Manual No. M86-090-J0105. Assembly & operating instructions, Small sample adapter. Brookfield Engineering laboratories Inc. Middleboro, USA

165. PerkinElmer. Sulphur and Phosphorous analysis in vegetable oil and beef tallow for biodiesel production using the Optima Inductively Coupled Plasma – Optical emission Spectrometer. Written by: Hilligoss D., IMS Analytical services, Ltd. and Ostrovsky M., PerkinElmer Life and Analytical Sciences.

166. AOCS (1998). Official methods and recommended practices of the AOCS. American Oil Chemists' Society. *Oils and Fats Analysis*. 5th edition. The society, 1998

167. http://www.chembuddy.com/?left=CASC&right=density_tables Accessed 27/2/2010

168. Minitab Inc. Minitab. State College PA 16801 – 3008, USA, Version 15.

169. ISO 8217: 2005 (E). Petroleum products – Fuels (class F) – Specifications of marine fuels. International Standard. Third edition. 2005-11-01

170. King J. M., Prinyawiwatkul W., Sathivel S. and Yin H. (2009). Comparisons of chemical and physical properties of catfish oils prepared from different extracting processes. *Journal of food science*, **74**, 2, E70 – E76.

171. Sathivel S. Presentation on: Oil from fish processing byproducts and underutilized fish as a viable renewable resource for biodiesel production. Fishery and industrial technology center. University of Alaska Fairbanks, Kodiak, Alaska.

172. Delucchi M. A. (2003). An Appendix to the report "A lifecycle emissions model (LEM): Life cycle emissions from transportation fuels, motor vehicles, transportation modes, electricity use, heating and cooking fuels, and materials. Appendix A: Energy use and emissions from the life cycle of diesel-like fuels derived from biomass. Institute of Transportation studies, University of California, USA

173. Canakci M. and Van Gerpen J. H. (2003). Comparison of engine performance and emissions for petroleum diesel fuel, yellow grease, biodiesel and soybean oil biodiesel. *Transactions of the ASAE*. **46**, 4, 937.

174. http://dodgeram.org/tech/dsl/FAQ/diesel_fuel.htm Accessed 28/3/2010

175. Augustin M. A., Burgar I., Shen Z. and Udabage P. (2005). Characterization of fish oil-in-water emulsions using light-scattering, nuclear magnetic resonance, and gas chromatography-headspace analysis. *Journal of American Oil Chemists' Society*, **11**, 797 – 802.

176. Doisaki N., Kohata T., Nakaya K., Ohshima T. and Ushio H. (2006). Effect of oil droplet sizes of oil-in-water emulsion on the taste impressions of added tastants. *Fisheries Science*, **72**, 4, 877-883

177. Presentation on: Energy Reduction Seafood Processing Sector. Sponsored by; Newfoundland and Labrador Department of Fisheries and Aquaculture, Feb 22-24 2010

178. Maidment G. G. and Prosser G. (2000). The use of CHP and absorption cooling in cold storage. *Applied Thermal Engineering*, **20**, 1059-1073

179. http://www.plantservices.com/articles/2005/530.html Accessed 1/3/2010

180. http://books.google.ca/books?id=iEVoOy0TrYsC&pg=PA41&lpg=PA41&dq=industrial+centrifuge+torque&source=bl&ots=AQq8jNWZIY&sig=IqRlZFgluMeE6g6lEj4xLBAcDgU&hl=en&ei=DA6HS_CZIIeY8Ab_-NWlDw&sa=X&oi=book_result&ct=result&resnum=3&ved=0CBgQ6AEwAg#v=onepage&q=torque&f=false Accessed 2/3/2010

181. AGS (2007). Particle emissions from ships. The Alliance for Global Sustainability. By: Dr. Sarah Janhall, Göteborg Atmospheric centre, department of chemistry, Göteborg University, Göteborg.

182. http://www.spe.org/industry/reference/unit_conversions.php Accessed 27/2/2010

183. http://www.engineeringtoolbox.com/air-properties-d_156.html Accessed 2/3/2010

184. Ahumada R., Contreras S., and Rudolph A. (2004). Evaluation of coastal waters receiving fish processing waste: Lota bay as a case study. *Environmental monitoring and assessment*, 90, 89-99

185. Islam M. S. Khan S. and Tanaka M. (2004). Waste loading in shrimp and fish processing effluents: potential sources of hazards to the coastal and nearshore environments. Science Direct, *Marine Pollution Bulletin*, **49**, 103-110