Journal of Food Processing & Technology
ISSN: 2157-7110

Deepika, et al,. J Food Process Technol 2014, 5:12
DOI: 10.4172/2157-7110.1000401

Research Article Open Access

# Investigation on Oil Extraction Methods and its Influence on Omega-3 Content from Cultured Salmon

**Deepika D[1]*, Vegneshwaran VR[1], Julia P[1], Sukhinder KC[2], Sheila T[1], Heather M[1] and Wade M[1]**

[1]*Centre for Aquaculture and Seafood Development, Marine Institute of Memorial University of Newfoundland, St. John's, Newfoundland, Canada*
[2]*Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada*

## Abstract

Salmon by-products are excellent source of polyunsaturated fatty acids, especially omega-3 and omega-6. The efficiency of different oil extraction techniques from salmon gut, head and frame including enzymatic (30°C for 2 h and 40°C for 4 h) and heat (90°C for 20 min) were compared and the influence on the quality of oil for nutraceutical applications was investigated. The highest oil yield was obtained from the salmon gut (80.01%), head (59.92%) and frame (78.58%) samples treated with enzyme at 30°C for 4 h. The chemical properties including peroxide value, p-anisidine value, TOTOX value, free fatty acid content and acid value were evaluated to determine the salmon oil quality using different methods. The peroxide value (0.28-2.65 meq/kg), p-anisidine value (0.16-1.03), TOTOX value (0.71-10.73), free fatty acid (0.17-1.06%) and acid value (0.33-2.10 mg/KOH g) of all oil samples extracted at different temperature and reaction time were within the recommended limits except for the higher peroxide value (5.26 meq/kg) for the oil extracted from head at 90°C using heat and higher free fatty acid content (1.67-6.49%) and acid value (3.32-17.49) for the oil extracted from the gut samples. The higher peroxide value was due to interaction between iron-containing protein (myoglobin) and lipid membrane which is released during heat treatment and induces oxidation of lipids. The higher free fatty acid content and acid value in the oil extracted from gut was because of the presence of endogenous enzymes in the gut which causes rapid autolysis of gut tissues during processing and oil extraction. The oils extracted using different methods were analyzed for the fatty acid content. The salmon gut, head and frame oil contains saturated fatty acids (19.21-21.93 g/100 g), monounsaturated fatty acids (36.82-40.17 g/100 g) and polyunsaturated fatty acids (38.89-39.83 g/100 g). The total omega 3 and omega 6 fatty acids present in the salmon gut, head and frame was in the range of 23.41-25.73 g/100 g and 10.27-12.03 g/100 g, respectively. The ratio of omega 3/omega 6 fatty acids present in the salmon gut, head and frame was in the range of 1.96-2.50 g/100 g. The ratio of DHA/EPA present in the salmon gut, head and frame was in the range of 0.95-1.07 g/100 g. The oil extracted using enzymatic methods had slightly higher fatty acid content than the heat extracted oil. The preliminary quality analysis of the oil extracted from different salmon parts at different temperatures and reaction times and the presence of higher EPA, DPA and DHA suggested that the oil had good quality standards; it is not oxidized and can be subjected to various nutraceutical applications.

**Keywords:** Endogenous enzymes; Fatty acid; Nutraceutical; Omega-3 fatty acids

## Introduction

Canadian aquaculture production is categorized into two main fishes: finfish and shell fish. The total aquaculture production in Canada was 174,057 tonnes worth $833,822 in 2012. Farmed salmon is one of the most important grown species in the Canadian aquaculture industry. About 108,118 tonnes (62.11%) worth $598,845 was produced in 2012. Newfoundland and Labrador produced 16,831 tonnes of Salmonids worth $99,286 in 2012 [1]. Other major species based on the production volume includes of mussels (16.15%), oysters (6.42%) and trout (3.73%). Canada accounts for 8.2% of global salmon production and ranks fourth behind Norway, The United Kingdom and Chile. British Columbia and New Brunswick account for 66.59 and 27.94% of the total salmon production, respectively [2].

There are 77 registered fish processing facilities in Newfoundland, ranging in size from feeder plants (processing fish to the fillet) to large year-round plants (processing fish into various fresh and frozen products including secondary processing) [3]. Of the 155 licensed aquaculture plants, 87 produced salmonids [4]. During fish processing operations significant amount of discards including skin, frame and trimmings (gut, fins, tail) were produced which can be used as a great source to generate various value added products such as fish oil, proteins, amino acids, biodiesel and omega-3 fatty acids [5]. Generally, fish contains 2-25% fat, 15-30% protein and 50-80% moisture content. Atlantic salmon contains 2-15% fat content and 57% of the total body fat is present in the inedible portion while skin contains 18% of the total body fat [6]. Fish oil is comprised of saturated, monounsaturated and polyunsaturated fatty acids. Polyunsaturated fatty acids are essential lipids which cannot be synthesized by mammalian organisms and therefore these fatty acids must be consumed from an external source. Fish oils are the one of the major sources of long chain polyunsaturated fatty acids including cis-5,8,11,14,17-Eicosapentaenoic Acid (EPA) and cis-4,7,10,13,16,19-Docosahexaenoic Acid (DHA) [7].

Generally, lipids are classified into two major groups: neutral or non-polar lipids and polar lipids. The neutral or non-polar lipids are comprised of Triacylglycerols (TAG), Diacylglycerols (DAG), Monoacylglycerols (MAG) and sterols. The polar lipids are comprised of Free Fatty Acids (FFA), Phospholipids (PL) and sphingolipids. Fish tissue is mainly composed of triacylgylcerols which are present in hydrophobic aggregates and contain fatty acids of varying chain lengths and different degree of unsaturation [8].

Lipid extraction is usually carried out using simple organic extraction techniques such as soxhlet (petroleum ether), chloroform-

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

**Received** October 30, 2014; **Accepted** November 24, 2014; **Published** December 03, 2014

**Citation:** Deepika D, Vegneshwaran VR, Julia P, Sukhinder KC, Sheila T, et al. (2014) Investigation on Oil Extraction Methods and its Influence on Omega-3 Content from Cultured Salmon. J Food Process Technol 5: 401. doi:10.4172/2157-7110.1000401

methanol extraction (using either Folch or Bligh and Dyer technique) and acid hydrolysis. Fish tissues contain structural lipids which are predominantly phospholipids that are closely attached to the proteins and other biopolymers. Complete extraction of these phospholipids is very essential due to presence of a high percentage of polyunsaturated fatty acids which cannot be achieved using petroleum ether extraction techniques. Chloroform-methanol extraction technique is used for complete extraction of lipids from fish tissue, however it is time consuming, requires highly skilled technician for accurate results and there are disposal issues with used solvent. Acid hydrolysis technique can also be used to extract lipids but it is very aggressive and extracts are chemically degraded and unsuitable for fatty acid profiling [9,10]. Various commercially available cost-effective food grade enzymes such as alcalase, neutrase, lecitase ultra, protex and protamex have been used for oil extraction via enzymatic hydrolysis. Generally, the enzymatic process is carried out under mild conditions (temperature: 40-60°C; pH 5-8) for shorter periods of time (30-120 min). In addition, superior quality food grade protein hydrolyzate can also be produced as a by-product during enzymatic oil extraction [11-13].

The main aim of this study was to evaluate the oil extraction methods (via heat and enzymatic route) from of salmon by-products (gut, head and frame) and compare the quality of salmon oil for nutraceutical application. The specific objectives were:

(a) To study the effectiveness of reaction temperature (30 and 40°C) during enzymatic oil extraction

(b) To study the effectiveness of reaction time (2, 4 h) during enzymatic oil extraction

(c) To compare the oil yield and omega-3 composition while using the heat and enzymatic route for salmon oil extraction.

## Materials and Methods

### Sample collection and preparation

Whole fish were collected in Styrofoam boxes on ice from a salmon aquaculture processing plant, Newfoundland, Canada and shipped overnight to the Marine Bioprocessing Facility, Marine Institute of the Memorial University of Newfoundland, St. John's, NL, Canada. The fish were hand-filleted and three waste streams (by-products) including (Figure 1): gut, frame/trimmings and head were collected separately and homogenized in a Hobart™ meat grinder without addition of water. The homogenized material was placed in the cooler (2-8°C) until oil extraction was performed. Oil extraction from these by-products was completed within two days of homogenization.

### Chemicals and enzymes

The Sea-B-Zyme L200 enzyme used in this study was obtained from Speciality Enzymes, Chino, California, United States of America. The chemicals used in this study include: 95% ethanol purchased from East Chem, Paradise, Newfoundland, Canada; phenolphthalein indicator, sodium hydroxide, acetic acid, chloroform, isooctane and p-anisidine reagent purchased from Fisher Scientific, Ottawa, Ontario, Canada; potassium iodide purchased from VWR International, Mississauga, ON, Canada; and sodium thiosulfate purchased from Sigma-Aldrich, Oakville, Ontario, Canada. The reagents used in this study include: 0.1 N sodium hydroxide, 0.025 N sodium hydroxide and 0.01 N sodium thiosulfate with 100 mL distilled water.

### Experimental procedure

**Enzymatic hydrolysis:** The enzymatic extraction of oil was carried out according to the procedure described in Figure 2. A minced fish sample (1000 g) was weighed and placed in four 500 mL glass bottles and 100 mL of water was added to the fish in the ratio of 1:0.4 (fish: water) and mixed well using a magnetic stirrer (Corning Magnetic Stirrer PC 210, Thermo Scientific, Marietta, Ohio, USA). The pH of the mixture was measured using a pH meter (Orion 5 Star pH meter, Thermo Scientific, Billerica, Massachusetts, USA) and adjusted to 6 by adding 1N HCl. All glass bottles were then placed in a water bath shaker (Precision 2870 Series, Thermo Scientific, Marietta, Ohio, USA) operating at 140 rpm and 30°C and kept for 30 min. The temperature was measured using a thermometer. The enzymatic hydrolysis was started by adding 0.5% (by weight of raw material) Sea-B Zyme L200 to each bottle. After hydrolysis for 4 h, the mixture was taken and placed in another water bath (Precision 280 Series, Thermo Scientific, Marietta, Ohio, USA) operating at 90°C for 5 min to inactivate the enzymes. The mixture was then allowed to cool and centrifuged (Beckman centrifuge, Thermo Scientific, Marietta, Ohio, USA) at 7500 g for 15 min. Four layers were formed in the centrifuge tubes: upper oil layer, light lipid layer, soluble clear protein layer and bottom sludge layer containing the remaining fish tissues, respectively. The upper oil layer was removed using a pipette and 0.1% (w/v) BaynoxTM antioxidant solution was added and stored at -30°C until further analysis. The same experimental procedure was performed at 40°C for 2 h and the oil yield was compared between the two different extraction protocols. Three replicates were carried out for both enzymatic extraction protocols.

**Heat extraction:** Heat extraction was carried out from salmon byproducts as described in Figure 3. A minced fish sample (1000 g) was weighed and placed in four 500 mL glass bottle and 100 mL of water was added to the fish in the ratio of 1:0.4 (fish : water) and mixed well using a magnetic stirrer (Corning Magnetic Stirrer PC 210, Thermo Scientific, Marietta, Ohio, USA). For heat extraction, the mixture was heated at 90°C on a hot plate under continuous stirring and kept at this temperature for 20 min. The mixture was then allowed to cool and centrifuged (J2-21M/E Beckman centrifuge, Thermo Scientific, Marietta, Ohio, USA) at 7500 g for 15 min. Three layers were formed in the centrifuge tubes: upper oil layer, soluble clear protein layer and bottom sludge layer containing the remaining fish tissues, respectively. The upper oil layer was removed using a pipette and 0.1% (w/v) BaynoxTM antioxidant solution was added and stored at -30°C until further analysis.

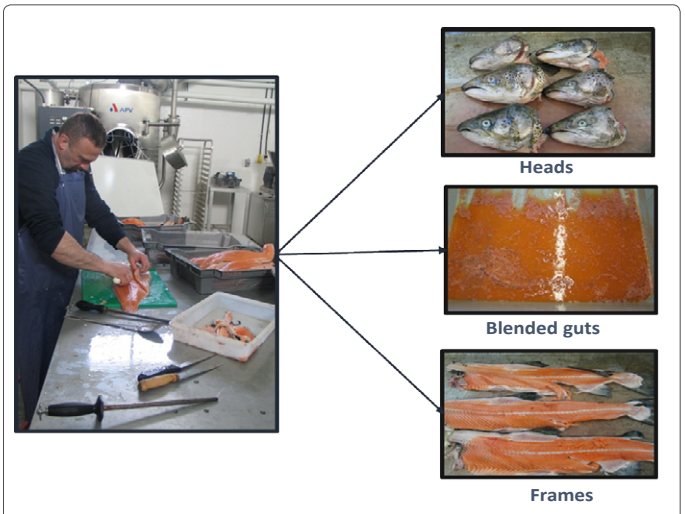



**Figure 1:** Illustration of byproducts produced after filleting salmon

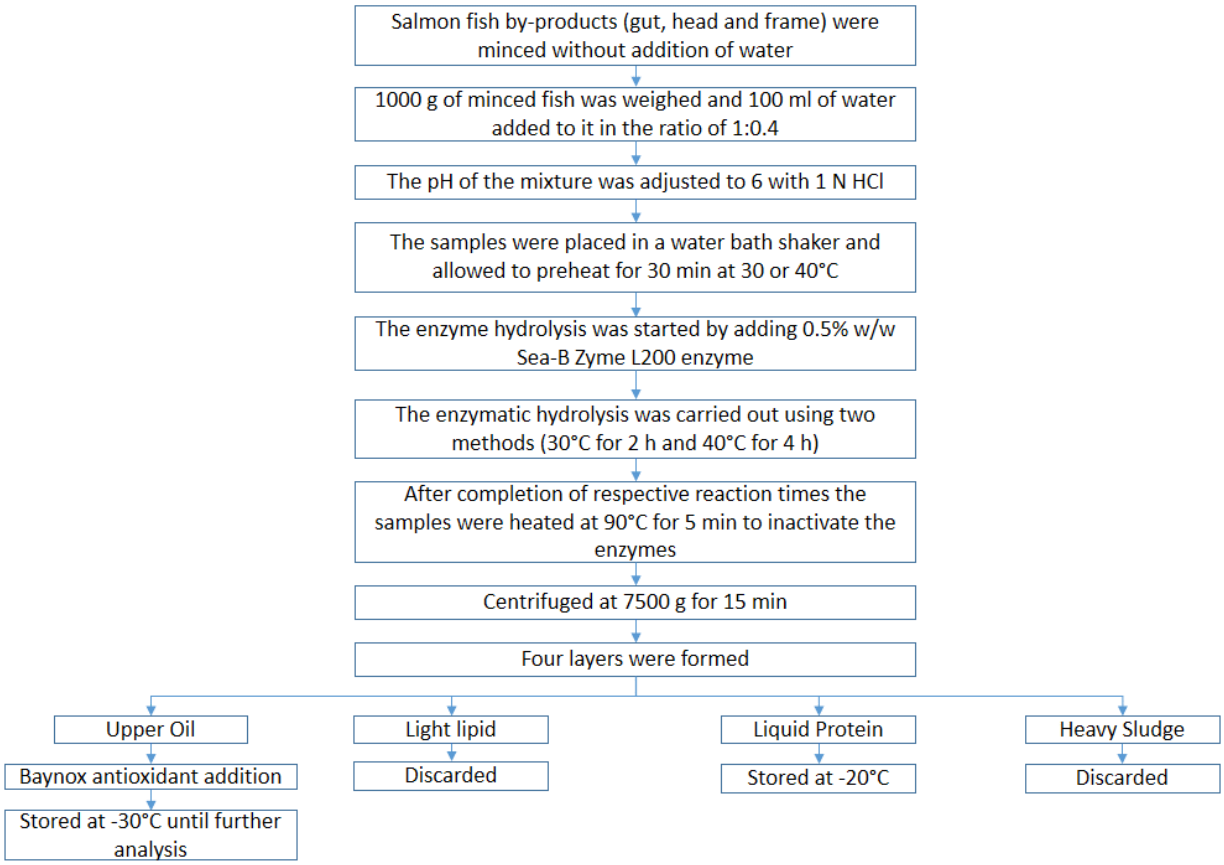



**Figure 2:** Experimental protocol for salmon by products oil extraction via enzymatic hydrolysis.

***Proximate analysis of fish samples***

Moisture content was determined using AOAC Official Method 938.08 by placing 2 g of fish sample into a pre-weighed aluminum dish. Samples were then dried in a hot air convection oven at 105°C overnight or until a constant weight was reached [14]. The total crude protein content of fish samples was determined by AOAC Official Method 940.25 using the Kjeldahl nitrogen method (N X 6.25) [15]. Total lipids in each sample were extracted using a cellulose thimble and hexane in combination with soxhlet extraction apparatus as described by Aryee and Simpson. The ash content of the fish samples was determined by AOAC Official Method 938.08 by charring approximately 2 g of sample in a crucible over a Bunsen burner and then heating in a muffle furnace at 550°C until the ash had a white appearance [16].

***Oil quality***

Smell, colour and physical state

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

***Peroxide Value (PV)***

The peroxide value was determined by AOCS official method Cd 8-53. The test oil sample was filtered through a Whatman No. 40 filter paper to remove moisture and impurities. A 5 g sample of filtered oil was accurately weighed to 0.05 g in a 250 mL Erlenmeyer flask. 30 mL of 3:2 acetic acid-chloroform was added and swirled to mix well. Two blank samples were simultaneously prepared without the addition of fish oil. To the samples, 0.5 mL of saturated potassium iodide solution was added and allowed to stand for exactly 1 min. Saturated potassium iodide solution was prepared by adding 10 g potassium iodide to 6 mL boiled distilled water so that undissolved potassium iodide crystals were present during analysis. After the standing time 30 mL of distilled water was immediately added to the oil samples and swirled to mix. The samples were titrated against 0.1 N sodium thiosulfate until the yellow iodine color disappeared. Starch indicator (2 mL) was added and the titration was continued against 0.1 N sodium thiosulfate until the blue color disappeared. The blank titration value must not exceed 0.1 mL and the peroxide value was calculated by using Equation 1. Preliminary results showed a titration value less than 0.5 mL, therefore, the peroxide value determination was carried out using 0.01 N sodium thiosulfate [17].

$$Peroxide\,Value\left(milliequivalents\ peroxide\,/\,1000\,g\ sample\right)=\frac{(S-B)*N*1000}{W}$$

Where:

S = Volume of titrated sample (mL)

B = Volume of titrated blank (mL)

N = Normality of sodium thiosulfate solution

W = Weight of oil (g) p-Anisidine value

***p-anisidine value***

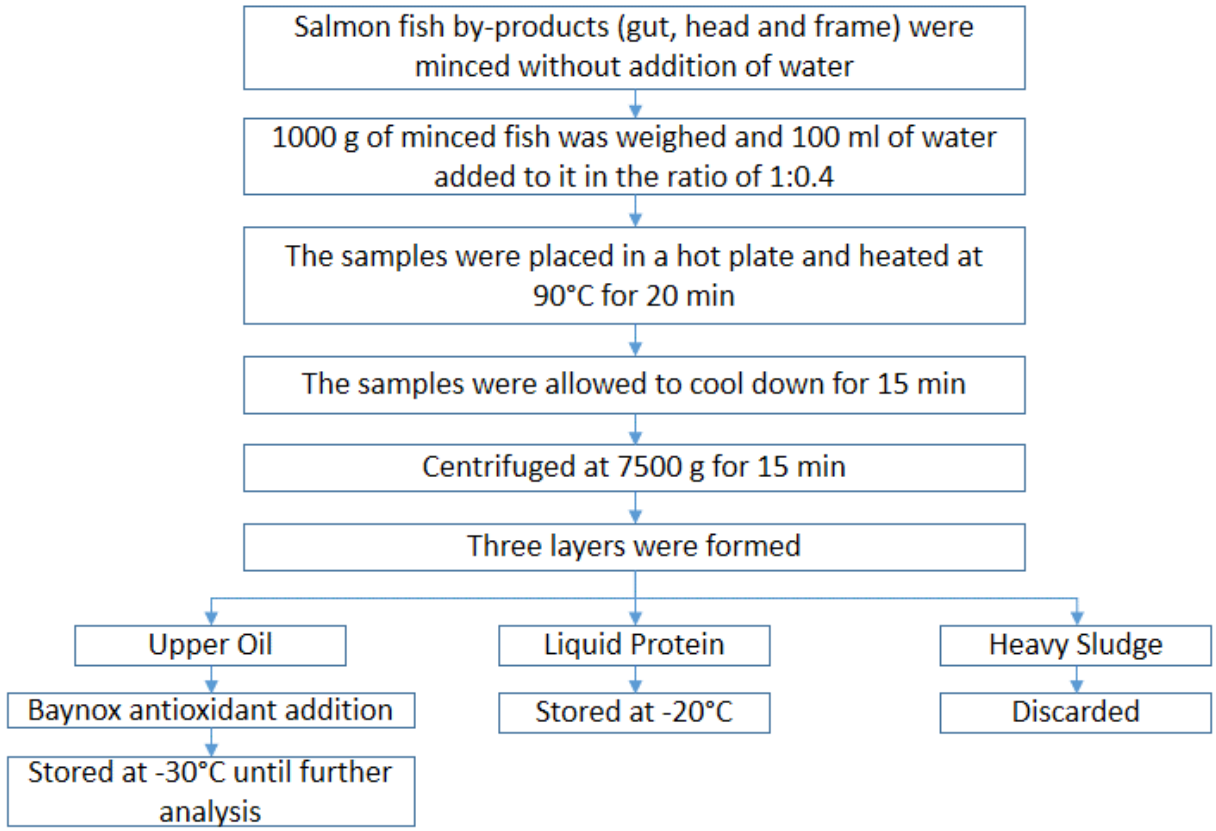



**Figure 3:** Experimental protocol for salmon by products oil extraction via heat extraction.

p-anisidine value was determined by AOCS official method Cd 18-90. The test oil sample was filtered through a Whatman No. 40 filter paper to remove moisture and impurities. A 0.5-4 ± 0.001 g sample of oil was accurately weighed in a 25 mL volumetric flask. The oil samples were then dissolved and diluted with 25 mL iso-octane. The absorbance (AB) of the oil sample was measured at 350 nm using a spectrophotometer (Jenway 6400/6405, Jenway Incorporated, Stone, Staffordshire, UK). A 5 mL sample of oil was pipetted into one test tube and 1 mL of p-anisidine reagent was added. 5 mL of iso-octane was added to another test tube with 1 mL of p-anisidine reagent and used as a blank. The p-anisidine reagent was prepared by adding 0.25 g p-anisidine to 100 mL of glacial acetic acid. After 10 minutes, the absorbance (AS) of the oil sample with the p-anisidine reagent was immediately measured at 350 nm using a spectrophotometer. The p-anisidine value was calculated by using following Equation 2 [18].

$$p-anisidine\,value=\frac{25*\left(1.2A_S-A_B\right)}{W}$$

Where:

AS = Absorbance of the fat solution after reaction with the p-anisidine reagent

AB = Absorbance of the fat solution

W = Weight of oil (g)

***TOTOX value***

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

***Free Fatty Acids and Acid Number***

The free fatty acid content (%FFA) and Acid number (AN) were determined according to AOCS Official method Ca 5a-40. A well-mixed oil sample (7.05 ± 0.05 g) was accurately weighed into a 250 mL Erlenmeyer flask and 75 mL of hot neutralized 95% ethanol and 2 mL of 1% phenolphthalein indicator solution were added to the oil sample. The hot neutralized 95% ethanol was prepared by heating 75 mL of 95% ethanol with 2 mL of 1% phenolphthalein indicator solution to incipient boiling. The ethanol was neutralized by adding 0.25N sodium hydroxide solution until a faint permanent pink color appeared. The oil samples were then titrated against 0.25N sodium hydroxide until the appearance of the first permanent pink color of the same intensity as that of the neutralized ethanol before the addition of sample. The permanent pink color persisted for at least 30 seconds during titration. The Free Fatty Acids content (%FFA) and acid number were calculated using Equations 3 and 4 [19]:

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

Where:

N = Normality of NaOH solution

W = Weight of oil (g)

Acid number (mgKOH/g)=1.99 X FFA (%)

## Fatty acid Analysis of Oil

***Lipid extraction***

Lipid samples were extracted according to Parrish [20]. An aliquot of 250 µl of oil (170-215 mg) sample was weighed in a test tube containing 2 mL of chloroform. Previous to the addition of the oil sample, the test tubes and Teflon® lined caps were rinsed 3 times with methanol and chloroform. 1 mL of ice-cold methanol, 1 mL of 2:1 chloroform: methanol and 0.5 mL of chloroform extracted water were added to the test tube. Chloroform extracted water was prepared by adding 1L of distilled water and 30 mL of chloroform to a separating funnel. The funnel was manually shaken for 2 minutes; the chloroform was allowed to settle and was then removed from the bottom of the funnel. This procedure was repeated twice to remove any lipids present in the distilled water. The test tube was then recapped and sonicated for 10 minutes followed by centrifugation for 2-3 minutes at 3000 rpm using an international clinical centrifuge (model CL, International Equipment Co, Needham, Mass). The entire lower organic lipid layer was removed by a double pipetting technique and transferred to a 15 mL vial that was cleaned 3 times with methanol and chloroform, respectively [21]. The double pipetting technique was performed in three steps. First, an ashed 14 cm pipette was passed through the top aqueous layer in the test tube, by bubbling air with the pipette bulb to prevent the aqueous layer from entering the 14 cm pipette until it touched the bottom of the test tube. Second, the pipette bulb was removed and a 27 cm pipette was placed inside the shorter pipette until it touched the bottom of the test tube. Third, the lipid layer was removed using the long pipette and transferred to a second cleaned 15 mL vial. Each of the short and long pipettes was washed with 3 mL ice-cold chloroform and the wash was collected, subsequently. The samples were again resonicated, centrifuged, double pipetted and the pipettes were rinsed three times as previously described and all the organic layers were pooled together. The extracted lipid was then evaporated under a gentle stream of nitrogen, sealed with Teflon® tape and stored in the freezer at -20°C until use.

***Preparation of fatty acid methyl esters (FAME) with $H_2SO_4$ in MeOH***

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

***FAME analysis using gas chromatograph***

An aliquot of 10 µL of the mixture was separated into fatty acid class based on the carbon atom by a gas chromatography system (HP6890 Series II, Agilent Technologies, Mississauga, Ontario, Canada), coupled with Flame Ionization Detector (FID) and 7683 auto sampler. A ZB wax+ polar capillary column 30 m in length, 0.32 mm of internal diameter and 0.25 µm film thicknesses (Phenomenex, Torrance, CA, USA) was used for analyses. The separated samples were injected directly into the column with an initial oven temperature of 65°C for 5 minutes, followed by ramping to a temperature of 195°C at a rate of 40°C /min for 15 minutes and again ramping to a final temperature of 22°C at a rate of 2°C /min. A final temperature of 220°C was held for 0.75 minutes. The detection system was equipped with a Flame Ionization Detector (FID) operating at 260°C with hydrogen as a carrier gas at a flow rate of 2 mL/min. The injector temperature was started at 150°C and ramped to a final temperature of 250°C at a rate of 120°C /minute. Peaks were identified using retention times from standards purchased from Supelco: 37 component FAME mix (Product number 47885-U), Bacterial acid methyl ester mix (product number 47080-U), PUFA 1 (product number 47033) and PUFA 3 (product number 47085-U). Chromatograms were integrated using the Varian Galaxie Chromatography Data System, version 1.9.3.2. The total run time was 32 minutes.

## Results and Discussion

### Weight distribution and nutritional composition

The average weight of a whole salmon fish was 4766.66 g. The gut, head, frame/trimmings and fillet make up 557.83 g (11.70%), 504.16

| Fish | Whole Fish (g) | Gut (g) | Head (g) | Frame (g) | Fillet (g) |
|---|---|---|---|---|---|
| 1 | 5000 | 575 | 507 | 780 | 3400 |
| 2 | 5000 | 609 | 499 | 800 | 3400 |
| 3 | 4400 | 474 | 457 | 522 | 3000 |
| 4 | 5000 | 601 | 620 | 622 | 3400 |
| 5 | 4800 | 573 | 484 | 670 | 3000 |
| 6 | 4400 | 515 | 458 | 612 | 2800 |
| Total | 28600 | 3347 | 3025 | 4006 | 19000 |
| Average | 4766.66 | 557.83 | 504.16 | 667.66 | 3166.66 |
| (%) | 100 | 11.70 | 10.57 | 14.00 | 66.43 |

**Table 1:** Weight distribution of salmon fish

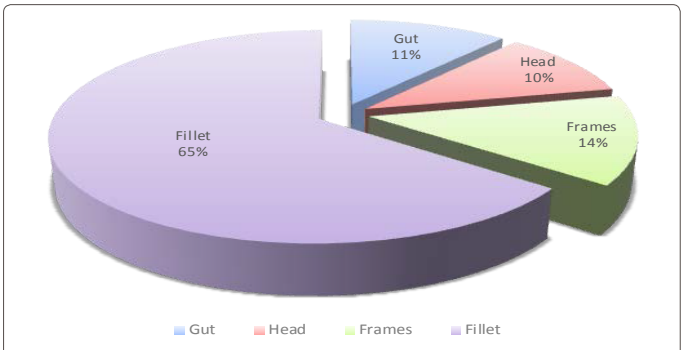



**Figure 4:** Average distribution of edible portion and by products in salmon

| Sample | Moisture (%) | Protein (%) | Lipid (%) | Ash (%) |
|---|---|---|---|---|
| Gut | 60.455 | 10.385 | 22.12 | 1.94 |
| Head | 63.355 | 11.31 | 21.86 | 3.515 |
| Frame/trimmings | 57.185 | 16.36 | 22.65 | 3.645 |

**Table 2:** Nutritional composition of salmon by-products

g (10.57%), 667.66 g (14.00%) and 3166.66 g (66.4%), respectively as shown in Table 1 and Figure 4.

The nutritional composition (moisture, protein, lipid and ash content) of salmon fish by-products including gut, head and frame/trimmings are shown in Table 2. The average moisture, protein, lipid and ash contents were 60.45, 10.385, 22.12 and 1.94% for the gut, 63.355, 11.31, 21.86 and 3.515% for the head and 57.185, 16.36, 22.65 and 3.645% for the frame/trimmings, respectively. The frame/trimmings had the highest protein (16.36%), lipid (22.65%) and ash contents (3.645%) while the head had the highest moisture content (63.55%).

## Oil yield

Fish oil was extracted from the gut, head and frame via heat and enzymatic extraction technique. After centrifugation during enzymatic hydrolysis, four layers (Figures 5 and 6) were formed in the centrifuge tubes: upper oil layer, light lipid layer, soluble clear protein layer and bottom sludge layer containing the remaining fish tissues. This occured during both experiments. However, compared to enzymatic hydrolysis at 30°C for 4 h, fewer solids settled at the bottom of the tube, indicating a more complete hydrolysis at 40°C for 2 h. In addition to the above mentioned four layers, very soft muddy phase was also observed just above the bottom sludge layer (slightly visible as black layer in Figure 6a) during gut hydrolysis. Mbatia et al. [11] reported that enzymatic hydrolysis resulted in formation of four phases including: an oily phase, emulsion phase, aqueous phase (protein hydrolysate) and sludge while recovering oil recovered from salmon head using 0.5% (w/w) Bromelain enzyme at 55°C for 14 h without addition of water or pH adjustment. After heat extraction, three layers (Figure 7) were formed in the centrifuge tubes: upper oil layer, soluble clear protein layer and bottom sludge layer containing the remaining fish tissues, respectively. Overall, heat extraction was the easiest process in terms of handling. Due to the higher temperatures, all meat protein became denatured and flocculated. The protein flocs and the solids (bones) were settled at the bottom of the centrifuge tube and remained attached to the tube. There was no middle phase between oil and soluble clear protein layer. Three replicates were carried out for heat extraction protocols. After enzymatic hydrolysis and heat extraction, fish oil obtained by centrifugation was weighed and oil yield was calculated and the results are shown in Table 3 and Figures 8 and 9. The results indicated that at 30°C and 4 h, the oil yield from salmon gut, head and frame were 80.01, 59.92 and 78.58%, respectively. When the oil extraction was carried out at 40°C and 2 h, the oil yield from salmon gut, head and frame were 76.40, 57.18 and 75.49%, respectively. The results from heat extraction indicated that the oil yield from salmon gut, head and frame were 79.11, 48.03 and 79.47%, respectively. In the present study, the highest oil yield was obtained from the salmon samples treated with enzyme at 30°C for 4 h. The results were similar to those reported by Mbatia et al., Liaset et al., Gbogouri et al. and Linder et al. [11,12,22,23].

Mbatia et al. [11] extracted oil from salmon head using 0.5% (w/w) bromelain enzyme at 55°C for 14 h without addition of water

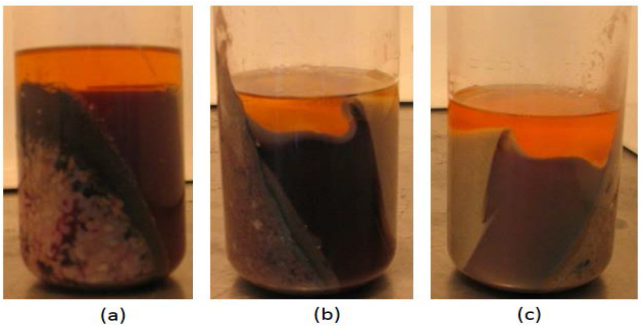



**Figure 5:** Four layers formed when recovering salmon oil from (a) gut (b) head (c) frame/trimmings during enzymatic hydrolysis at 30°C for 4 h.

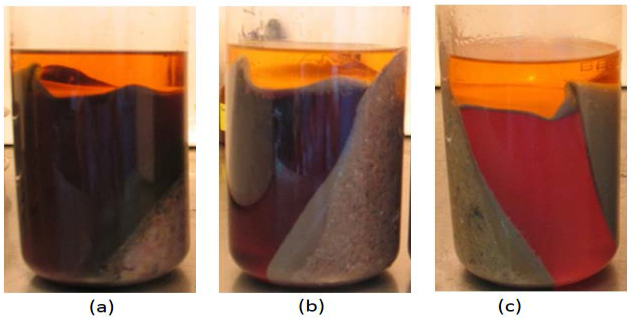



**Figure 6:** Four layers formed when recovering salmon oil from (a) gut (b) head (c) frame/trimmings during enzymatic hydrolysis at 40°C for 2 h.

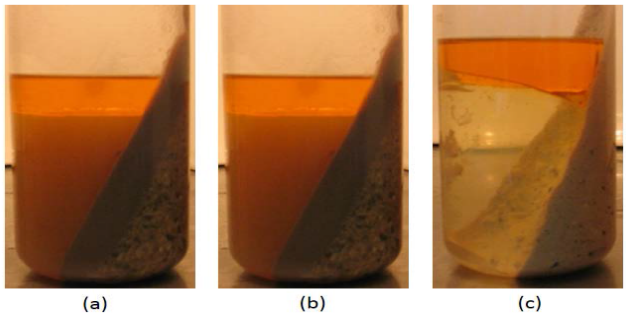



**Figure 7:** Three layers formed when recovering salmon oil from (a) gut (b) head (c) frame/trimmings during heat extraction at 90°C for 20 min.

| Weight (g) | Time (min) | Temperature (°C) | Oil Yield | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Gut | | Head | | Frame | |
| | | | (g) | (%) | (g) | (%) | (g) | (%) |
| 1000 | 240 | 30[E] | 177 | 80.01 | 131 | 59.92 | 178 | 78.58 |
| 1000 | 120 | 40[E] | 169 | 76.40 | 125 | 57.18 | 171 | 75.49 |
| 1000 | 20 | 90[H] | 175 | 79.11 | 105 | 48.03 | 180 | 79.47 |

*E=Enzyme

**Table 3:** Oil extraction from salmon gut, head and frames using enzyme and heat.

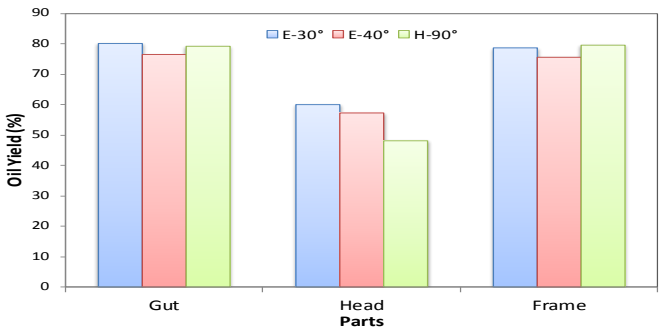
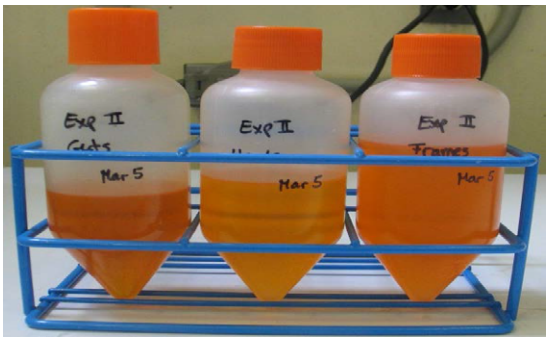
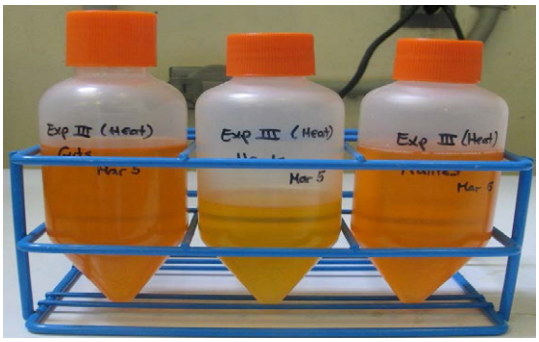



**Figure 8:** Oil yield from salmon parts using enzyme and heat extraction techniques (E-Enzyme; H-Heat).


(a) Enzymatic hydrolysis at 30°C for 4 h




(b) Enzymatic hydrolysis at 40°C for 2 h




(c) Heat extraction at 90°C for 20 min

**Figure 9:** Recovered salmon oil from gut, head and frame/trimmings (left to right) via enzymatic hydrolysis and heat extraction.

or pH adjustment and reported a maximum oil yield of 69.3% after 2 h of hydrolysis. They also reported that increasing the time from 4 to 14 h did not increase the oil yield and also resulted in degradation of polyunsaturated fatty acids due to the formation of aldehydes and ketones. Addition of water during hydrolysis had a negative impact on the process as it resulted in the formation of an emulsion and the most of the lipids were lost in the emulsion.

Liaset et al. [22] extracted oil from 200 kg fresh salmon frame using 0.24 kg Protamex at a pH of 6.5, a temperature of 55°C for 1 h and reported the total oil yield of 38.7 kg (77.4%). The study also indicated that enzymatic hydrolysis resulted in aqueous fraction (0.6%), insoluble fraction (2.8%), bone fraction (2%) and emulsion (1.6%) contained lipids. The total amount of lipids obtained after enzymatic hydrolysis of salmon frame was 84.4%.

Gbogouri et al. [23] performed enzymatic hydrolysis of oil from salmon head (500 g) using three different enzymes (including: Neutrase 0.8L (5% w/v, 50°C, pH 7), Protamex (5% w/v, 50°C, pH 7.5) and Alcalase 2.4 L (5% w/v, 55°C, pH 8) for 2 h), solvent extraction and heat extraction processes. The results from enzymatic hydrolysis indicated that the oil recovery yield from salmon head using Neutrase, Protamex and Alcalase enzymes were 14.4, 14.6 and 19.6% (w/w), respectively. The solvent extraction and heat extraction processes resulted in 21.5 and 14.5% (w/w) oil yield, respectively. The oil yield from the enzymatic hydrolysis method was better than traditional heat extraction method since at the higher heat extraction (95°C) the lipids can be trapped inside packed unfolded proteins reducing oil release.

Linder et al. [12] extracted oil from 10 kg salmon head (20% total lipid content) using three enzymes (Neutrase (0.05% w/w, 45°C, pH 7), Alcalase (0.05% w/w, 55°C, pH 7.5) and Flavourzyme (0.05% w/w, 50°C, pH 7.5) for 2 h) and Bligh and Dyer solvent extraction. The results indicated that the oil recovery yield from salmon head using Neutrase, Alcalase and Flavourzyme enzymes were 17.2, 17.4 and 17.0% (w/w), respectively whereas the solvent extraction process resulted in a 20% oil recovery yield.

Analysis of variance (ANOVA) was performed on the oil yield data as shown in Table 4. The effects of fish parts and method were significant at the 0.001 level. The interactions among the various parameters were also significant at the 0.001 level.

The results of the Tukey's grouping are shown in Table 5. The oil yield from enzymatic hydrolysis method at 30°C (E-30) was significantly different from enzymatic hydrolysis method at 40°C (E-40) and heat extraction method at 90°C (H-90) at the 0.05 level. However, the oil yield from enzymatic hydrolysis method at 40°C (E-40) and heat extraction method at 90°C (H-90) were not significantly different from each other at the 0.05 level. The highest oil yield of 72.80% was obtained from the enzymatic hydrolysis method at 30°C. The oil yield from gut and frame were not significantly different from each other at the 0.05 level. However, the oil yield from head was significantly different from gut and frame at the 0.05 level. The highest oil yield of 78.70% was obtained from gut.

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| **Method** | 2 | 69.31 | 69.31 | 34.65 | 43.77 | 0.000 |
| **Parts** | 2 | 3223.47 | 3223.47 | 1611.74 | 2035.46 | 0.000 |
| **Method*Parts** | 4 | 194.25 | 194.25 | 48.56 | 61.33 | 0.000 |
| **Error** | 18 | 14.25 | 14.25 | 0.79 | | |
| **Total** | 26 | 3501.29 | | | | |

DF: Degree of freedom
SS: Sum of square
MS: Mean of square
EC: Enzyme concentration
RT: Reaction time
$R^2$: 99.59%

**Table 4:** Analysis of variance for oil yield.

| Factors | Level | N | Mean Yield (%) | Tukey Grouping |
|---|---|---|---|---|
| **Method** | E-30 | 9 | 72.80 | A |
| | E-40 | 9 | 69.9 | B |
| | H-90 | 9 | 69.0 | B |
| **Parts** | Gut | 9 | 78.70 | A |
| | Frame | 9 | 77.90 | A |
| | Head | 9 | 55.10 | B |

Groups with the same letter are not significantly different from each other at the 0.05 level.
E-30: Enzymatic hydrolysis at 30°C
E-40: Enzymatic hydrolysis at 40°C
H-90: Heat extraction at 90°C

**Table 5:** Tukey grouping on oil yield.

| Analysis | Enzymatic 30°C, 4 h | | | Enzymatic 40°C, 2 h | | | Heat Extraction 90°C, 20 min | | |
|---|---|---|---|---|---|---|---|---|---|
| | Gut | Head | Frame | Gut | Head | Frame | Gut | Head | Frame |
| Colour | Orange | Yellow | Orange | Orange | Yellow | Orange | Orange | Yellow | Orange |
| Odour | Fresh oil odour, not fishy | Fresh oil odour, not fishy | Fresh oil odour, not fishy | Fresh oil odour, not fishy | Fresh oil odour, not fishy | Fresh oil odour, not fishy | Fresh oil odour, not fishy | Fresh oil odour, not fishy | Fresh oil odour, not fishy |
| Peroxide value (meq/kg) | 1.57 | 0.49 | 0.48 | 1.45 | 0.28 | 0.28 | 2.65 | 5.26 | 0.42 |
| p-Anisidine value | 0.67 | 0.17 | 0.22 | 1.03 | 0.15 | 0.17 | 0.81 | 0.21 | 0.16 |
| TOTOX value | 3.81 | 1.15 | 1.18 | 3.93 | 0.71 | 0.73 | 6.11 | 10.73 | 1.00 |
| Free fatty acid (%) | 6.49 | 1.06 | 0.79 | 8.76 | 0.78 | 0.51 | 1.67 | 0.17 | 0.59 |
| Acid value (mg/KOH g) | 12.91 | 2.10 | 1.57 | 17.49 | 1.55 | 1.01 | 3.32 | 0.33 | 1.17 |

**Table 6:** Salmon oil quality analysis

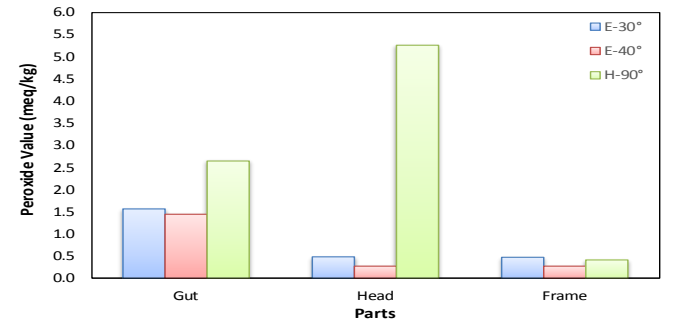



**Figure 10:** Peroxide value of the oil extracted from salmon by products via enzymatic hydrolysis and heat extraction

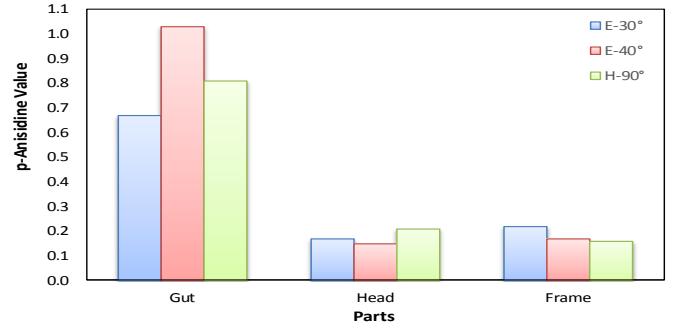



**Figure 11:** p-Anisidine value of the oil extracted from salmon by products via enzymatic hydrolysis and heat extraction

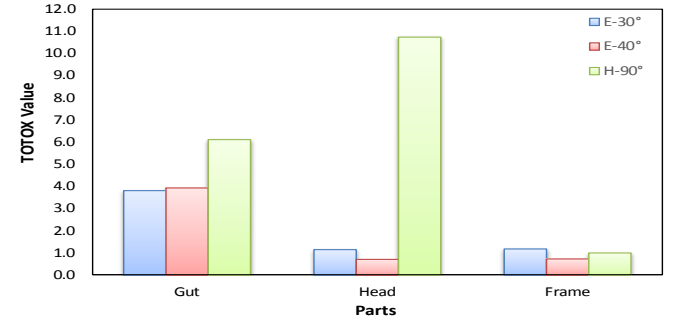



**Figure 12:** TOTOX value of the oil extracted from salmon by products via enzymatic hydrolysis and heat extraction

## Oil quality analysis

The oil extracted from salmon parts (gut, head and frame) was analysed for peroxide value, p-anisidine value, free fatty acid content, acid value and TOTOX value to determine the quality of the oil and the results are shown in Table 6.

## Peroxide value

Primary oxidation of oil and the measurement of hydroperoxides are determined by peroxide value analysis [24]. The allowable limit of peroxide value set by the Global Organization for EPA and DHA (GOED) and Food and Agricultural Organization of the United Nations (FAO) for quality and acceptability of fish oils for human consumption is ≤5 meq/kg [25]. The results from the present study indicated that the peroxide value of all oil samples extracted at different temperature and reaction time were between 0.28-2.65 meq/kg except for the oil samples extracted from head sample at 90°C (5.26 meq/kg) which is above the recommended limit of ≤5 meq/kg (Figure 10). Fish oils contain large amounts of polyunsaturated fatty acids (PUFA) and the maximum peroxide value can be reached during the early stages of oxidation due to the instability and rapid decomposition of hydroperoxides (primary oxidation product) into secondary oxidation products. Therefore, even if a fish oil has a lower peroxide value, can also be in an oxidized state. There are several factors including: lipid class composition, concentration of oxygen, light and presence of antioxidants that influence the formation of hydroperoxides and degradation into secondary oxidation products [26,27]. The hydroperoxides formed from n-3 polyunsaturated fatty acid decompose more rapidly than hydroperoxides formed from the fatty acids with a lesser degree of saturation. Under heating and in the presence of metals, the hydroperoxides decompose rapidly to form aldehydes, ketones, acids, esters, alcohols and short chain hydrocarbons. Therefore, even if a fish oil has a lower peroxide value it is not guaranteed to be a high quality fish oil because it may also be in an oxidized state. These hydroperoxides (primary oxidation products) do not affect the flavor of the oil [28]. Ritter et al. [29] analyzed 16 commercially available fish oil and reported peroxide values of the oil samples ranged between 1-14.8 meq/kg. In this study, the peroxide value of the oil samples extracted from the heat extraction was higher than the enzyme extracted oil samples. Chantachum et al. [30] reported that oil extracted from heat treated fish samples had a high peroxide value (25 mg/kg). During heat treatment, the iron-containing protein (myoglobin) denatures and iron gets released into the catalytic pool. These iron containing proteins interact with the lipid membrane and induces oxidation of lipids.

## p-Anisidine value

Secondary oxidation of oil and the measurement of aldehydes with α- and β-unsaturation are determined by p-anisidine value. The allowable limit of anisidine value set by Global Organization for EPA and DHA (GOED) and Food and Agricultural Organization of the United Nations (FAO) for quality and acceptability of fish oils for human consumption is ≤20 [31]. The results from the present study indicated that the anisidine values of all oil samples extracted at different temperatures and reaction times were between 0.16-1.03 which is below the allowed limit of 20 (Figure 11). Aidos et al. [32] reported an anisidine value of 8.9±0.5 from herring oil obtained from by-products. They documented that hydroperoxides are very unstable and decompose rapidly into volatile and non-volatile secondary oxidation products (high molecular weight saturated and unsaturated carbonyl compounds in triglycerols) which results into the rancid oil. Pak [33] analysed the stability and quality of fish oil during domestic application and reported an anisidine value of 19.8 and the addition of an antioxidant did not have an effect on the anisidine value during storage for 42 days. Boran et al. [34] studied the changes in the quality of garfish, golden mullet, shad and mackerel oils due to storage temperature and time and the anisidine value ranged from 1.74 to 14.09.

## TOTOX value

The total oxidation value (TOTOX) is a quality parameter used to determine the presence of various compounds such as hydroperoxides, aldehydes and ketones which are generated by degradation of polyunsaturated fatty acids under pro-oxidant conditions including high temperatures, oxygen, metal compounds and light [35]. The allowable limit of TOTOX value set by Global Organization for EPA and DHA and Food and Agricultural Organization of the United Nations (FAO) for quality and acceptability of fish oils for human consumption is ≤26. The results from the present study indicated that the anisidine value of all oil samples extracted at different temperatures and reactions times were between 0.71-10.73 which is within the allowed limit of 26 (Figure 12). Aidos et al. extracted oil from herring by-products and reported a TOTOX value of 14.9. Pak analysed the stability and quality of fish oil during domestic application and reported a TOTOX value of 21. Boran et al. analyzed the quality of four fish oils (garfish, golden mullet, shad and mackerel) using a number of chemical analyses, including Peroxide Value (PV) and Anisidine Value (AV). Based on the PV and AV, the calculated TOTOX values for the four fish oils ranged from 8.04 to 35.29. The higher TOTOX values for some fish oils were due to the extended storage time and temperature.

## Free fatty acid

Fish and fish tissues possess relatively high autolytic activities and high polyunsaturated fatty acid content which are prone to both lipolysis and oxidation. Therefore, the oils extracted from fish have a high free fatty acid content which is problematic during omega-3 extraction or biodiesel production. According to Bimbo [36], the allowable limit of free fatty acids for crude fish oil is in the range of 1-7% but usually 2-5%. In the present study, oil extracted from salmon head and frame using different extraction methods had lower free fatty acid contents (0.17-1.06%), whereas the free fatty acid of the oil extracted from salmon gut were between 1.67-6.49% (Figure 13). The presence of higher free fatty acid content in gut samples was due to the presence of endogenous enzymes causing autolysis of gut tissues during processing and oil extraction. Huang and Sathivel [37] reported that the free fatty acid

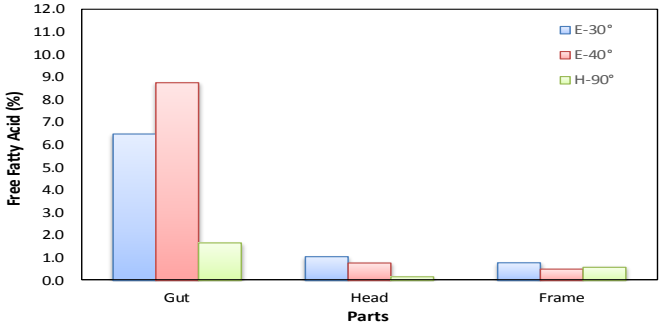



**Figure 13:** Free fatty acid value of the oil extracted from salmon by products via enzymatic hydrolysis and heat extraction

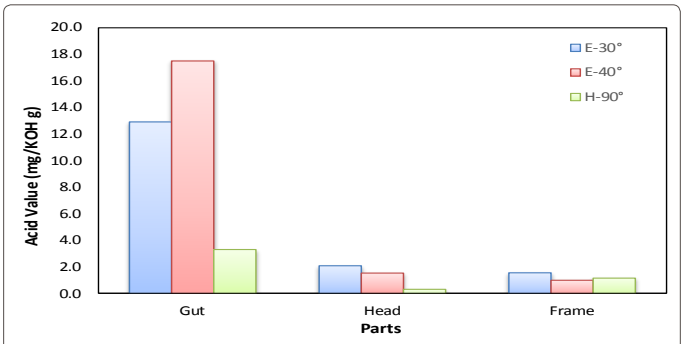



**Figure 14:** Acid value of the oil extracted from salmon by products via enzymatic hydrolysis and heat extraction

content of unpurified salmon oil was 3.5%. Kaitaranta [38] reported that the free fatty acid content of capelin oil was 3.2%. Chantachum et al. reported that oils prepared at higher temperatures have higher free fatty acid content because the hydrolysis of ester bonds of triglycerides is greater at higher temperature.

## Acid value

The amount of free fatty acids determines the acidity of the oil. The acid value is defined as the amount of KOH (mg) required to neutralize one gram of oil or fat and an increase in the free fatty acid content is directly proportional to an increase in the acid value [39,40]. The acceptable limit for acid value is 7-8 mg/KOH g. In the present study, oil extracted from salmon head and frame using different extraction methods had lower acid values (0.33-2.10 mg/KOH g) which are within the acceptable limit of 7-8 mg/KOH g. However, the acid values of the oil extracted enzymatically at 30°C and 40°C from salmon gut were 12.91 and 17.49 mg/KOH g (Figure 14), respectively which is above the acceptable limit of 7-8 mg/KOH g. Boran et al. reported that an increase in acid value is due to the lipase activity present in the microorganisms or fish tissue. Wrolstad et al. [41] reported that the acid value of the oil depends upon several factors including: oil composition, extraction procedure, sample preparation and freshness of raw material.

## Fatty acid analysis

The salmon oil extracted using three different protocols from three different salmon parts were subjected to fatty acid analysis to determine the amount of monounsaturated, saturated and polyunsaturated fatty acids present in the oil and the results are shown in Tables 7 and 8. The results indicated that the salmon gut, head and frame contain saturated fatty acids (19.21-21.93 g/100 g), monounsaturated fatty acids (36.82-39.58 g/100 g) and polyunsaturated fatty acids (38.89-39.83 g/100 g).

| Type | Isomer | Common Name | Gut | | | Head | | | Frame | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | E30 | E40 | H90 | E30 | E40 | H90 | E30 | E40 | H90 |
| Saturated Fats (SFA) | C 14:0 | Myristic acid | 3.99 | 4.00 | 3.95 | 3.95 | 4.00 | 4.05 | 4.09 | 4.15 | 4.10 |
| | C 15:0 | Pentadecanoic acid | 0.27 | 0.26 | 0.26 | 0.27 | 0.28 | 0.28 | 0.26 | 0.28 | 0.27 |
| | C 16:0 | Palmitic acid | 11.17 | 10.99 | 11.12 | 13.16 | 13.15 | 13.13 | 12.74 | 12.65 | 12.63 |
| | C 17:0 | Heptadecanoic acid | 0.57 | 0.54 | 0.57 | 0.64 | 0.63 | 0.60 | 0.54 | 0.59 | 0.60 |
| | C 18:0 | Stearic acid | 3.12 | 3.13 | 3.16 | 3.57 | 3.54 | 3.55 | 3.35 | 3.32 | 3.32 |
| | C 20:0 | Arachidic acid | 0.17 | 0.19 | 0.16 | 0.14 | 0.17 | 0.19 | 0.18 | 0.17 | 0.16 |
| | C 22:0 | Behenic acid | 0.12 | 0.11 | 0.13 | 0.12 | 0.12 | 0.12 | 0.10 | 0.11 | 0.12 |
| Total SFA | | | **19.40** | **19.21** | **19.35** | **21.86** | **21.88** | **21.93** | **21.26** | **21.27** | **21.21** |
| Monounsaturated Fats (MUFA)<br>Total MUFA<br>Polyunsaturated Fats (PUFA) | C 14:1 | Myristoleic acid | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.02 | 0.03 | 0.03 |
| | C 16:1 n-9 | Cis-7 hexadecenoic acid | 0.28 | 0.23 | 0.26 | 0.30 | 0.34 | 0.29 | 0.31 | 0.29 | 0.30 |
| | C 16:1 n-7 | Palmitoleic acid | 6.32 | 6.27 | 6.22 | 6.27 | 6.17 | 6.25 | 6.43 | 6.42 | 6.36 |
| | C 17:1 | Heptadecenoic acid | 0.15 | 0.16 | 0.18 | 0.14 | 0.15 | 0.16 | 0.16 | 0.17 | 0.17 |
| | C 18:1 n-9 | Oleic acid | 23.56 | 24.11 | 24.50 | 21.57 | 21.98 | 21.96 | 21.98 | 22.35 | 22.32 |
| | C 18:1 n-7 | Vaccenic acid | 3.39 | 3.33 | 3.36 | 3.41 | 3.41 | 3.40 | 3.38 | 3.39 | 3.39 |
| | C 20:1 n-11 | Gadoleic acid | 0.27 | 0.28 | 0.28 | 0.26 | 0.27 | 0.32 | 0.28 | 0.29 | 0.28 |
| | C 20:1 n-9 | Gondoic acid | 2.52 | 2.48 | 2.58 | 2.25 | 2.41 | 2.37 | 2.29 | 2.29 | 2.29 |
| | C 20:1 n-7 | Paullinic acid | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.29 | 0.27 | 0.27 | 0.28 |
| | C 22:1 n-9 | Erucic acid | 0.37 | 0.36 | 0.38 | 0.38 | 0.35 | 0.37 | 0.35 | 0.34 | 0.35 |
| | C 22:1 n-11 | Cetoleic acid | 2.05 | 2.05 | 2.11 | 1.93 | 2.09 | 2.09 | 1.88 | 1.97 | 2.00 |
| | | | **39.22** | **39.58** | **40.17** | **36.82** | **37.48** | **37.53** | **37.34** | **37.81** | **37.77** |
| | C 16:2 n-4 | Hexadecadienoic acid | 0.78 | 0.77 | 0.77 | 0.76 | 0.76 | 0.76 | 0.79 | 0.79 | 0.78 |
| | C 18:2 n-6 | Linoleic acid | 9.71 | 10.15 | 10.20 | 8.48 | 8.67 | 8.62 | 8.71 | 9.03 | 9.00 |
| | C 18:3 n-4 | Linolenic acid | 0.40 | 0.39 | 0.38 | 0.37 | 0.36 | 0.34 | 0.38 | 0.39 | 0.36 |
| | C 18:3 n-3 | Alpha-linolenic acid | 2.10 | 2.16 | 2.14 | 1.75 | 1.74 | 1.76 | 1.81 | 1.86 | 1.86 |
| | C 18:3 n-6 | Gamma-linolenic acid | 0.21 | 0.22 | 0.21 | 0.21 | 0.21 | 0.20 | 0.22 | 0.21 | 0.22 |
| | C 18:4 n-3 | Stearidonic acid | 1.06 | 1.06 | 1.05 | 1.10 | 1.06 | 1.07 | 1.11 | 1.11 | 1.12 |
| | C 18:4 n-1 | Alpha-parinaric acid | 0.53 | 0.53 | 0.51 | 0.55 | 0.53 | 0.53 | 0.58 | 0.53 | 0.54 |
| | C 20:2 n-6 | Eicosadienoic acid | 0.63 | 0.64 | 0.65 | 0.60 | 0.57 | 0.59 | 0.62 | 0.59 | 0.60 |
| | C 20:3 n-6 | Dihomo-gamma-linolenic acid | 0.27 | 0.29 | 0.25 | 0.24 | 0.25 | 0.25 | 0.23 | 0.24 | 0.26 |
| | C 20:4 n-3 | Eicosatetraenoic acid | 1.15 | 1.16 | 1.10 | 1.10 | 1.06 | 1.00 | 1.04 | 1.07 | 1.08 |
| | C 20:4 n-6 | Arachidonic acid | 0.69 | 0.74 | 0.64 | 0.75 | 0.76 | 0.70 | 0.73 | 0.65 | 0.72 |
| | C 20:5 n-3 | Eicosapentaenoic acid (EPA) | 7.70 | 7.64 | 7.41 | 8.88 | 8.67 | 8.68 | 8.81 | 8.69 | 8.65 |
| | C 22:5 n-3 | Docosapentaenoic acid (DPA) | 4.30 | 4.23 | 4.19 | 4.02 | 3.87 | 3.88 | 4.16 | 4.03 | 4.02 |
| | C 22:6 n-3 | Docosahexaenoic acid (DHA) | 8.25 | 7.79 | 7.53 | 8.90 | 8.49 | 8.45 | 8.55 | 8.21 | 8.22 |
| Other PUFA | | | 2.04 | 2.03 | 1.98 | 2.05 | 2.00 | 2.04 | 2.08 | 1.97 | 2.03 |
| Total PUFA | | | **39.81** | **39.80** | **39.01** | **39.74** | **39.00** | **38.89** | **39.83** | **39.36** | **39.44** |
| Other fatty acids | | | **1.56** | **1.41** | **1.46** | **1.58** | **1.64** | **1.66** | **1.56** | **1.56** | **1.58** |

*E=Enzyme
H=Heat

**Table 7:** Salmon oil fatty acid analysis (g/100 g of oil).

| Type | Gut | | Head | | | Frame | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E30 | E40 | H90 | E30 | E40 | H90 | E30 | E40 | H90 | Fillet |
| **Total Fatty Acid** | 98.44 | 98.59 | 98.54 | 98.42 | 98.36 | 98.34 | 98.44 | 98.44 | 98.42 | 16.3 |
| **Total Saturated Fatty Acids (SFA)** | 19.4 | 19.21 | 19.35 | 21.86 | 21.88 | 21.93 | 21.26 | 21.27 | 21.21 | 3.83 |
| **Total Monounsaturated Fatty Acids (MUFA)** | 39.22 | 39.58 | 40.17 | 36.82 | 37.48 | 37.53 | 37.34 | 37.81 | 37.77 | 5.7 |
| **Total Polyunsaturated Fatty Acids (PUFA)** | 39.81 | 39.8 | 39.01 | 39.74 | 39 | 38.89 | 39.83 | 39.36 | 39.44 | 5.94 |
| **Total Omega 3 Fatty acids** | 24.56 | 24.04 | 23.41 | 25.73 | 24.89 | 24.85 | 25.49 | 24.96 | 24.94 | 4.1 |
| **Total Omega 6 Fatty acids** | 11.5 | 12.03 | 11.95 | 10.27 | 10.46 | 10.36 | 10.51 | 10.73 | 10.8 | 1.83 |
| **Omega 3/Omega 6** | 2.13 | 2 | 1.96 | 2.5 | 2.38 | 2.4 | 2.43 | 2.33 | 2.31 | 2.24 |
| **DHA/EPA** | 1.07 | 1.02 | 1.02 | 1 | 0.98 | 0.97 | 0.97 | 0.95 | 0.95 | 0.85 |
| **Total EPA, DPA and DHA** | 20.24 | 19.66 | 19.13 | 21.79 | 21.03 | 21.01 | 21.52 | 20.93 | 20.89 | 3.73 |

*E=Enzyme
H=Heat

**Table 8**: Comparison of fatty acids present in salmon oil and fillet (g/100 g).

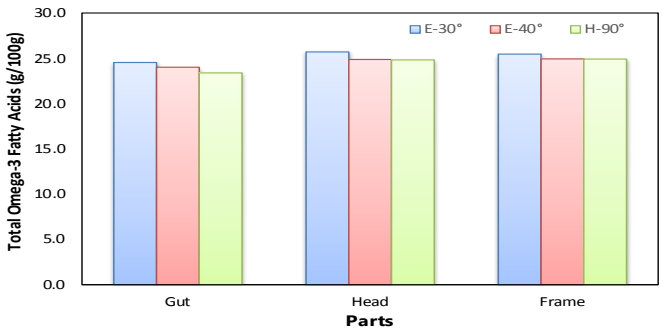



**Figure 15:** Total omega-3 fatty acid present in the oil extracted from salmon by products via enzymatic hydrolysis and heat extraction

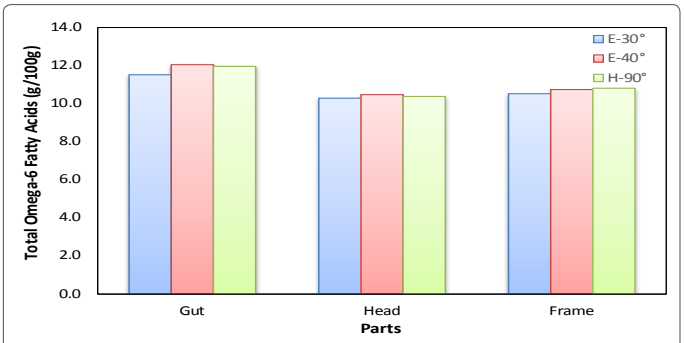



**Figure 16:** Total omega-6 fatty acid present in the oil extracted from salmon by products via enzymatic hydrolysis and heat extraction

The major saturated and monounsaturated fatty acid present in salmon gut, head and frame was palmitic acid (10.99-13.16 g/100 g) and oleic acid (21.57-24.50 g/100 g), respectively. The major polyunsaturated fatty acids present in the salmon gut, head and frame were linoleic acid (8.48-10.20 g/100 g), eicosapentaenoic acid (EPA) (7.41-8.88 g/100 g), docosapentaenoic acid (DPA) (3.87-4.30 g/100 g) and docosahexaenoic acid (DHA) (7.53-8.90 g/100 g). The total omega 3 and omega 6 fatty acids present in the salmon gut, head and frame were in the range of 23.41-25.73 g/100 g and 10.27-12.03 g/100 g, respectively (Figures 15 and 16). The ratios of omega 3/omega 6 fatty acids present in the salmon gut, head and frame were in the range of 1.96-2.50 g/100 . The results had demonstrated that omega-3 and omega-6 present in salmon fillet was 4-5 and 10-11 times lesser compared to salmon by-products (Table 8). The ratios of DHA/EPA present in the salmon gut, head and frame were in the range of 0.95-1.07 g/100 g. The results also indicated that the oil extracted using enzymatic methods had a slightly higher fatty acid content than the heat extracted oil. The results were similar to the studies reported by Gbogouri, Peng, Sun, and Kahveci et al. [42-44]. Gbogouri extracted oil from salmon head using solvent and enzyme and analysed the fatty acid composition of the oil. The results indicated that the oil extracted using solvent had saturated fatty acids (24.6%), monounsaturated fatty acids (39.9%) and polyunsaturated fatty acids (35.4%) while the oil extracted using Alcalase enzyme had saturated fatty acids (25.2%), monounsaturated fatty acids (40.8%) and polyunsaturated fatty acids (34%) in a slightly higher range. The total omega-3 fatty acids present in the solvent and enzyme extracted oil was 27.7 and 26.2%, respectively. The study suggested that enzymatic extraction method yielded competent quality oil in comparison to solvent and heat extraction methods. Peng et al. studied the fatty acid composition of triglyceride and phospholipid fractions of the oil extracted from two different Atlantic salmon species (anadromous and landlocked) and reported that the triglyceride fraction of the oil contained higher saturated (28.47%) and monounsaturated fatty acids (45.28%) compared to the saturated (24.42%) and monounsaturated fatty acid (42.36%) present in the phospholipid fraction of the oil. However, the polyunsaturated fatty acid (33.45%) present in the phospholipid fraction was higher than the polyunsaturated fatty acid (26.49%) present in the triglyceride fraction of the oil. The study also suggested that the marked difference in the polyunsaturated fatty acid content between the two species is attributed by several factors including: diet, genetic specificity and environmental salinity.

Sun et al. [43] extracted oil from farmed Atlantic salmon using 12 N hydrochloric acid for 12 h and the oil was analysed for fatty acid composition and quantification. The major fatty acids identified in the study included: myristic acid (7.59%), palmitic acid (19.21%), palmitoleic acid (11.49%), oleic acid (22.06%), eicosapentaenoic acid (7.91%) and docosahexaenoic acid (6.99%). The total saturated, monounsaturated and polyunsaturated fatty acids present in the salmon oil were 31.73, 36.05 and 32.22%, respectively. The study also suggested that the fatty acid profile of visceral oil was similar to the oil obtained from the fillet. Therefore, the oil extracted from viscera can be used in high grade functional food and feed and it also beneficial to the fish industry by adding value to the fish processing waste.

Kahveci et al. [44] extracted omega-3 fatty acids from salmon oil which was produced using an enzymatic hydrolysis process. The total saturated, monounsaturated and polyunsaturated fatty acids present in the salmon oil were 18.58, 48.2 and 30.68%, respectively. The enzyme extracted salmon oil contained eicosapentaenoic acid (4.8%), docosapentaenoic acid (2.04%) and docosahexaenoic acid (6.93%) [45].

## Conclusion

The present study was carried out to determine the efficiency of enzyme and heat to extract oil from salmon gut, head and frame and its effect on the yield, quality and omega-3 content present in the oil. The extraction method played a significant role on the amount of oil released from the fish tissue. The enzymatic hydrolysis at 30°C was superior to enzymatic hydrolysis at 40°C and heat extraction at 90°C. The highest oil yield was obtained from salmon gut extracted using enzyme at 30°C. The enzyme hydrolysis at low temperature was able to compete with the commercial heat extraction method and was able to give better quality oil than heat extracted oil. Therefore, enzymatic hydrolysis has the potential to be substituted as the cost effective and environment friendly oil extraction protocol instead of the heat extraction method. The oil obtained from salmon gut and frame was orange in color whereas the oil obtained from salmon head was yellow in color. The peroxide value of the oils extracted using enzyme at 30 and 40°C were within the allowed limit (5 meq/kg). However, the oil extracted using heat at 90°C was above the recommended limit suggesting that heat extraction might deteriorate oil quality. The anisidine and TOTOX value of all oil samples extracted at different temperatures and reaction times were within the allowed limit of 20 and 26, respectively. The free fatty acid content of the oil extracted from salmon head and frame was very low. However, the free fatty acid of the oil extracted from salmon gut was high (1.67-6.49%) due to the presence of endogenous enzyme causing autolysis of gut tissues during processing and oil extraction. The acid value of the oil extracted at different temperatures and reaction times were within the allowed limit of 7-8 mg/KOH g. The preliminary quality analysis of the oil extracted from different salmon parts at different temperatures and reaction times suggested that the oil had good quality standards and

it is not oxidized. The oil extracted from salmon gut, head and frame contains saturated fatty acids (19.21-21.93 g/100 g), monounsaturated fatty acids (36.82-40.17 g/100g) and polyunsaturated fatty acids (38.89-39.83 g/100 g). The oil is composed of higher polyunsaturated fatty acid content including: linoleic acid (8.48-10.20 g/100 g), Eicosapentanoic Acid (EPA) (7.41-8.88 g/100 g), Docosapentaenoic Acid (DPA) (3.87-4.30 g/100 g) and docosahexaenoic acid (DHA) (7.53-8.90 g/100 g). The omega-3 and omega-6 present in salmon by-products were 4-5 and 10-11 times higher compared to salmon fillet. The higher EPA, DPA and DHA present in the by-products oils suggests that the oil can be utilized for various nutraceutical applications.

### Acknowledgement

Financial support received from the Canadian Centre for Fisheries innovation (CCFI) and National Research Council of Canada (NRC) is gratefully acknowledged.

### References

1. CAIA (2014) Production and markets. Canadian Aquaculture Industry Alliance.
2. DFO (2014) Aquaculture: Production quantities and values. Department of Fisheries and Oceans Canada, Ontario, Canada.
3. DFA (2014a) Newfoundland and Labrador Fishing Industry Highlights 2012 and 2013 (Preliminary). Department of Fisheries and aquaculture, Newfoundland and Labrador.
4. DFA (2014b) Newfoundland and Labrador Aquaculture Industry Highlights 2012 and 2013. Department of Fisheries and aquaculture, Newfoundland and Labrador.
5. Ramakrishnan VV, Ghaly AE, Brooks MS, Budge SM (2013) Extraction of oil from mackerel fish processing waste using Alcalase enzyme. Enzyme Engineering 2: 1-10.
6. Aryee ANA, Simpson BK (2009) Comparative studies on the yield and quality of solvent extracted oil from salmon skin. Journal of Food Engineering 92: 353-358.
7. Hamilton MC, Hites RA, Schwager SJ, Foran JA, Knuth BA, et al. (2005) Lipid composition and contaminants in farmed and wild salmon. Environ Sci Technol 39: 8622-8629.
8. Fahy E, Cotter D, Sud M, Subramaniam S (2011) Lipid classification, structures and tools. Biochim Biophys Acta 1811: 637-647.
9. Johnson RB, Barnett HJ (2003) Determination of fat content in fish feed by supercritical fluid extraction and subsequent lipid classification of extract by thin layer chromatography-flame ionization detection. Aquaculture, 216: 263-282.
10. Ghaly AE, Ramakrishnan VV, Brooks MS, Dave D (2013) Fish processing wastes as a potential source of proteins, amino acids and oils: A critical review. Journal of Microbial & Biochemical Technology 5: 107-129.
11. Mbatia B, Adlercreutz D, Adlercreutz P, Mahadhy A, Mulaa F (2010) Enzymatic oil extraction and positional analysis of-3 fatty acids in Nile perch and salmon head. Process Biochemistry 45: 815-819.
12. Linder M, Fanni J, Parmentier M (2005) Proteolytic extraction of salmon oil and PUFA concentration by lipases. Mar Biotechnol (NY) 7: 70-76.
13. Slizyte R., Rustad T, Storro I (2005) Enzymatic hydrolysis of cod (Gadus morhua) by-products optimization of yield and properties of lipid and protein fractions. Process Biochemistry 40: 3680-3692.
14. AOAC Official Method 930.15 (2000) Moisture in Animal Feeds. American Oil Chemists Society, Champaign, Illinois, USA.
15. AOAC Official Method 938.08 (2000) Ash of Seafood. American Oil Chemists Society, Champaign, Illinois, USA.
16. AOAC Official Method 940.25 (2000) Nitrogen (Total) in Seafood. American Oil Chemists Society, Champaign, Illinois, USA.
17. AOCS Official Method Cd 8-53 (2003) Peroxides in fats and oils. American Oil Chemists Society, Champaign, Illinois, USA.
18. AOCS Official Method Cd 18-90 (1997) p-Anisidine value. Sampling and analysis of commercial fats and oils. American Oil Chemists Society, Champaign, Illinois, USA.
19. AOCS Official Method Ca 5a-40 (1997) Free Fatty Acids. American Oil Chemists Society, Champaign, Illinois, USA.
20. Parrish CC (1999) Determination of total lipid, lipid classes, and fatty acids in aquatic samples M.T. Arts. Lipids in Freshwater Ecosystems, Springer-Verlag, New York.
21. Hooper T, Parrish (2009) Profiling neutral lipids in individual fish larvae by using short column gas chromatography with flame ionization detection. Limnology and Oceanography: Methods 7: 411-418.
22. Liaset B, Julshamn K, Espe M (2003) Chemical composition and theoretical nutritional evaluation of the produced fractions from enzymatic hydrolysis of salmon frame with Protamex. Process Biochemistry 38: 1747-1759.
23. Gbogouri GA, Linder M, Fanni J, Parmentier M (2006) Analysis of lipids extracted from salmon (Salmo salar) head by commercial proteolytic enzymes. European Journal of Lipid Science and Technology 108: 766-775.
24. Dave D, Ramakrishnan VV, Trenholm S, Manuel H, Pohling J, et al. (2014) Marine Oils as Potential Feedstock for Biodiesel Production: Physicochemical Characterization. J Bioprocess Biotech 4: 2-12.
25. FAO/WHO (2013) Joint FAO/WHO food standards programme codex committee on fats and oils. Proposed draft standard for fish oils. Twenty third session, Langkawi, Malaysia.
26. Ritter JS (2012) Chemical measures of fish oil quality: oxidation products and sensory evaluation. Doctor of Philosophy Thesis Desertion, Dalhousie University, Halifax, Nova Scotia, Canada.
27. Sullivan JC, Budge SM (2010) Monitoring fish oil volatiles to assess the quality of fish oil. Lipid Technology 22: 230-232.
28. Kulås E, Ackman RG (2001) Different tocopherols and the relationship between two methods for determination of primary oxidation products in fish oil. J Agric Food Chem 49: 1724-1729.
29. Ritter JC1, Budge SM, Jovica F (2013) Quality analysis of commercial fish oil preparations. J Sci Food Agric 93: 1935-1939.
30. Chantachum S, Benjakul S, Sriwirat N (2000) Separation and quality of fish oil from precooked and non-precooked tuna head. Food Chemistry 69: 289-294.
31. GOED (2014) GOED Voluntary Monograph. Global Organization for EPA and DHA, Salt Lake City, Utah, United States of America.
32. Aidos I, van der Padt A, Boom RM, Luten JB (2001) Upgrading of maatjes herring byproducts: production of crude fish oil. J Agric Food Chem 49: 3697-3704.
33. Pak CS (2005) Stability and quality of fish oil during typical domestic application. Final Project, The United Nations Fisheries Training Programme, Iceland.
34. Boran G, Karacam H, Boran M (2006) Changes in the quality of ash oils due to storage temperature and time. Food Chemistry 98: 693-698.
35. Rubio-Rodriguez N, de Diego SM, Beltran S, Jaime I, Sanz MT (2012) Supercritical fluid extraction of fish oil from fish by-products: A comparison with other extraction methods. Journal of Food Engineering 109: 238-248.
36. Bimbo (1998) Guidelines for characterizing food grade fish oil. International Fish meal and Oil Manufacturers Association. Research report 9: 473-483.
37. Huang J, Sathivel S (2010) Purifying salmon oil using adsorption, neutralization and a combined neutralization and adsorption process. Journal of Food Engineering 96: 51-58.
38. Kaitaranta JK (1992) Control of lipid oxidation in fish oil with various antioxidative compounds. Journal of the American Oil Chemists Society 69: 810-813.
39. Rubio-Rodriguez N, de Diego SM, Beltran S, Jaime I, Sanz MT (2008) Supercritical fluid extraction of the omega-3 rich oil contained in hake (*Merluccius capensis–Merluccius paradoxus*) by-products: Study of the influence of process parameters on the extraction yield and oil quality. The Journal of Supercritical Fluids 47: 215-226.
40. Barthet VJ, Gordon V, Daun JK (2008) Evaluation of a colorimetric method for measuring the content of FFA in marine and vegetable oils. Food Chemistry 111: 1064-1068.
41. Wrolstad RE, Decker EA, Schwartz SJ (2005) Handbook of food analytical chemistry, water, proteins, enzymes, lipids, and carbohydrates. Science.
42. Peng J, Larondelle Y, Pham D, Ackman RG, Rollin X (2003) Polyunsaturated fatty acid profiles of whole body phospholipids and triacylglycerols in

anadromous and landlocked Atlantic salmon (Salmo salar L.) fry. Comp Biochem Physiol B Biochem Mol Biol 134: 335-348.

43. Sun T, Xu Z, Prinyawiwatkul W (2006) FA composition of the oil extracted from farmed atlantic salmon (*Salmo salar* L.) viscera. Journal of the American Oil Chemists Society 83: 615-619.

44. Kahveci D, Falkeborg M, Gregersen S, Xu X (2010) Upgrading of farmed salmon oil through lipase-catalyzed hydrolysis. The Open Biotechnology Journal 4: 47-55.

45. Sandy Brae Laboratories (2009) Water test kit instructions. Newark, Delaware, USA: Sandy Brae Laboratories Inc.