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Research Article

# Effect of Moisture Reduction and Harvest Times on Quality of Salmon Processing By-products

**Deepika Dave***, **Winny Routray**

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

***Corresponding author:** Deepika Dave, Marine Bioprocessing Facility, Centre of Aquaculture and Seafood Development, Marine Institute, Memorial University, St. John's, Newfoundland, Canada. Tel: +17097570732; Fax: +17097570742; Email: Deepika.Dave@mi.mun.ca

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## Abstract

This study assessed the storage quality of dried salmon by-products in terms of microbial quality, color and water activity and stored frozen salmon by-products in terms of oil and fatty acids yields. Microbial count and water activity were very low during the entire storage period in case of dried salmon by-products. The color of the dried sample didn't change significantly over the storage period. The oil yield of salmon frames harvested at different time periods increased with increase in storage period. Saturated fatty acids increased and Mono-Unsaturated Fatty Acids (MUFAs) decreased with increase in storage period. However, Poly-Unsaturated Fatty Acids (PUFA) and omega-3 fatty acids were least affected by the storage period.

**Keywords:** By-Product; Extraction; Fatty Acid; Quality; Salmon

## Introduction

Fish is a major food source for animal protein and several other health beneficial components including amino acids, enzymes, oils and fatty acids [1,2]. Worldwide production of fish has increased significantly, attributing to the increased demand of animal protein sources, which has subsequently increased the fish processing. Salmon (*Salmo salar*) is a popular fish, whose demand has increased immensely with increased demand of fish. Salmon processing removes the fillet portion of the salmon, but leaves the rest (45-50% of the body weight) as waste or for low-value uses. Salmon processing waste (by products) created environmental issues posed by limited disposal options including composting, animal feed and landfilling [3]. These salmon resources are a rich source of essential higher value biomolecules (nutraceuticals and pharmaceuticals) including: collagen, gelatin, protein, amino acids, bioactive peptides, omega-3s, oil and enzymes [4,5,6].

Fish biomaterial has high water content and lots of enzymes and therefore it is susceptible to rapid spoilage. The digestive enzymes present in the fish lead to autolysis causing solubilization of blood water containing both protein and oil. Also, other enzymes such as lipases can cause rapid deterioration leading to loss in quality and yield of fish oil. Oxygen and microbial spoilage also leads to rapid deterioration of fish tissues and therefore require special handling, handling, storage, preservation and pretreatment to minimize spoilage.

For efficient utilization of salmon waste resources and extraction of high value biomolecules, storage of these material under optimum conditions is very essential. Storage at low temperatures and under freezing conditions are common methods of preservation of biological materials. However, lipid peroxidation during freezing has been observed by Apgar, Hultin [7] in fish muscle microsomes. In past years, a lot of studies have been conducted for the development of optimum preservation techniques for increasing the shelf life of whole fish and fish based other products [6,8-10]; however, very few studies have reported oil, microbial and other quality parameters observed during storage of different forms of salmon- by-products [11]. Wu, Bechtel [11] studied the effect of storage on the quality of oil extracted from aged salmon heads and viscera, stored at 6 and 15°C for 4 days. They observed that even after 4 days of storage at 15°C, oil extracted from raw salmon heads and viscera continued to be a potential source of long chain omega-3 fatty acids; however, "Fat soluble antioxidant activity" decreased and free fatty acid levels further increased with increased storage temperature and time. However, there are no studies accounting the effect of storage on the quality of dried salmon by-products and/ or the effect of storage at freezing temperatures on the oil yield and composition of salmon oil extracted from frames.

Hence, the major objectives of this study were (a) to analyses the microbial quality, color and water activity of the dried salmon processing by-products under various storage conditions, and (b) to identify and quantify various fatty acids in salmon frame oil, harvested at various periods, employing enzymatic extraction.

## Materials and Methods

### Materials

All chemicals employed during the study were analytical grade. Sea-B-Zyme L200 enzyme, applied for all the enzymatic extraction processes was obtained from Speciality enzymes (Chino, California, USA). Dilution blanks (90 and 99 mL bottles of 0.1% peptone), Oxytetracycline Gentamicin Yeast extract (OGYE) and glucose agar purchased from Sigma-Aldrich (Oakville, Ontario, Canada)., hexane, phosphoric acid, sulfuric acid and sodium hydroxide, boric acid purchased from VWR International (Mississauga, ON, Canada).

### Preparation of Samples

The salmon (*Salmo salar)* processing waste applied during this whole study was obtained from local salmon processor, Newfoundland, Canada during the same year set (2014-2015). Each batch of the salmon was processed on same day of harvesting and salmon by-products were collected and supplied to Marine Bioprocessing Laboratories of Marine Institute, St John's, Newfoundland. Analytical studies reported in this manuscript were conducted on the salmon harvested at different intervals of the year. Proximate analyses were conducted with fish frames, heads and viscera fractions from the batch obtained on 30th November 2014 (Batch A), which were stored at 5˚C until further processing (48 hrs). Heads, frames and viscera were separately macerated with Hobart grinder, frozen, freeze dried, vacuum packed in polyethylene bags and stored at 5˚C for further analyses (Figure 1).

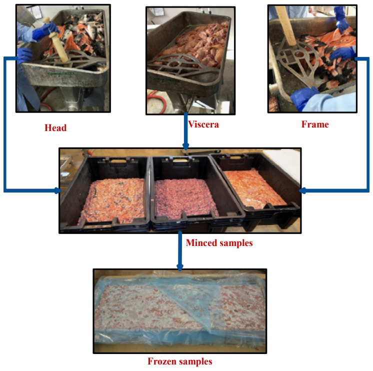

**Figure 1:** Illustration of byproducts sample preparation after filleting salmon.

Later, other experiments for study of storage quality of freeze dried salmon by-products were conducted with samples received on 27 February 2015, which were also stored in the refrigerator at 5˚C until processing (Batch B). First the salmon heads, viscera and frames were separately ground with Hobart grinder (Model 4146 The Hobart MFG. Co. Ltd.), with different blade sizes. Then equal weight of all the three ground samples were further finely ground using Ninja professional blender and mixed together. The mixed sample including ground head, viscera and frames was then laid on plates, frozen at -30˚C and freeze dried after 24 hours of freezing. The freeze drying was conducted in consecutive drying phases for maximum removal of moisture. The semidried pieces of fish samples were also broken into pieces for increasing the exposure of sample surface to the drying conditions leading to further removal of moisture from inside of the sample through the capillary effect. Freeze dried samples were vacuum packed in the polyethylene bags and stored at 5˚C and -30˚C in walk-in freezers, until further chemical and microbial analyses after 14 days, 28 days and 35 days.

Individually, salmon frames from the salmon by-products batches obtained at different periods during the same farming year (November 20, 2014; February 27, 2015; April 29, 2015 (Batch C); June 8, 2015 (Batch D)) of Atlantic salmon (*Salmo salar)* were applied for oil quality analyses. As mentioned before, samples obtained on November 20, 2014 and February 27, 2015 were ground after receipt and short storage at 5˚C, using Hobart grinder (Model 4146 The Hobart MFG. Co. Ltd.) with different blade sizes, and stored after vacuum packaging at -30˚C, until further processing and analysis. Samples were thawed in standard freeze for more than 24 hours before the enzymatic oil extraction and analyses. However, samples received later were vacuum packed and stored without any processing at -30˚C, until further analysis. Later these samples were thawed and ground with Ninja professional blender before enzymatic oil extraction.

### Proximate Analysis

Proximate analysis was conducted separately for individual fractions of fish head, frame and viscera of salmon by-products received in Batch A. Protein analysis was conducted using Kjeldahl method (AOAC 954.01). Lipid content was determined using the Soxhlet method (AOAC 948.15) and ash and moisture were determined according to standard dry ashing and moisture determination procedures (AOAC 938.08 and 930.15).

### Water Activity, Salinity and Colour

For the salmon by-products mixture prepared from Batch B, water activity was measured using AquaLab water activity meter (series 3/ 3TE, Decagon devices, Washington, USA) and colour was measured using ColorTec-PCM colorimeter (New Jersey, USA) using L, A and B scale. Salinity was measured using YSI Incorporated salinity meter (Model 30, Ohio, USA).

## Total Plate Count and Yeast and Mould Count

A homogenate of the freeze-dried salmon by-products mixture (Batch B) was prepared by combining 99mL of sterile diluent and 11 g of sample, which was homogenized for 2 min. Further serial dilutions of $10^{-2}$ to $10^{-7}$ were prepared "Using sterile peptone water dilution blanks and homogenized sample from the stomacher bag". For total yeast and mold count, about 15 to 20 mL of the agar was poured in the plates and were dried overnight at room temperature and stored at 5-10°C for future use. 0.1 mL aliquots of each dilution were transferred to the prepared OGYE plates and distributed evenly on the agar surface using a sterile bent glass rod. The plates were incubated in dark, for 5 days, after which colonies were count and reported as yeast and mold CFU/g. All the dilutions were tested in duplicates.

In case of Total Plate Count (TPC), for the analysis of total number of bacteria, 1 mL of the aliquot of each dilution was first poured into Petri plates, which was replicated. 12 to 15 mL of plate count agar (freshly prepared, sterilized using autoclave, and cooled to 44-46°C) was poured into the petriplates. The sample and agar were mixed and agar was allowed to solidify, after which the petriplates were inverted and incubated at 35°C for 48 hours. The colonies were counted and expressed as CFU/g.

## Enzymatic Extraction of Fish Oil and Fractionation of Digested Fish Biomaterial

A standard enzymatic method was developed based on our preliminary experiments and previous studies [12], which was applied for all the samples received at different periods of the farming year (Batch A, B, C and D) (Figure 2). Two hundred and fifty grams of ground fish frames was transferred into 1 L Erlenmeyer flask. Based on previous studies, 1.75 grams (which is 0.7% of total sample weight) of enzyme was mixed with 50 mL distilled water poured in a 100 mL measuring cylinder maintained at 30°C. An additional 50 mL of distilled water, maintained at 30°C was used for recuperating the enzyme from the measuring cylinder and added into the ground fish frame. The mixture with fish frames was digested in incubator shaker (Thermo- Scientific Max Q 6000, Marietta, Ohio, USA) set at 30°C and 100 rpm for 2 hours. Digestion was stopped by suspending the enzyme action through heat treatment at 70°C for 10 min. Two hundred mL of water was used for washing the bones and extracting maximum amount of digested fluid material into the centrifuge tubes and the digested mass was centrifuged at 3000 rpm for 15 mins, for separation of the digested mass into different fractions based on varying density.

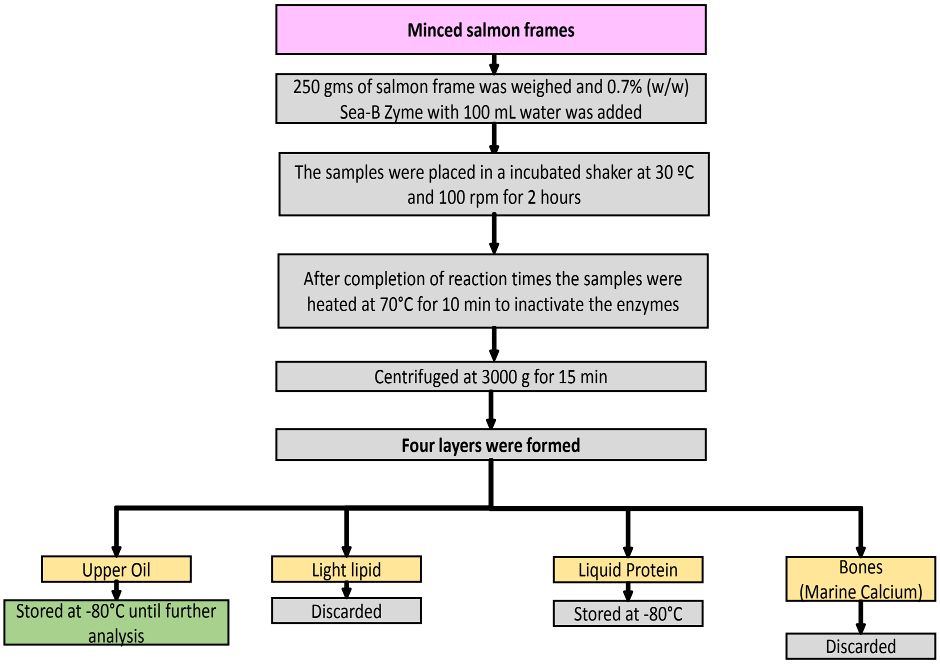

**Figure 2:** Experimental enzymatic oil extraction protocol from salmon frames.

The separated layers in the centrifuge tubes consisted of oil fraction on top, emulsion in the middle, protein fraction and solid material (Figure 3). Oil fraction and protein fraction were poured into the separatory funnel and retained for further separation of fluids. Protein fraction from the bottom was separated along with some dispersed oil fraction and the clear oil fraction was obtained. Protein and bone fraction were collected for separate research studies and solid material mixed with the emulsion (collected from the centrifuge tubes) were discarded.

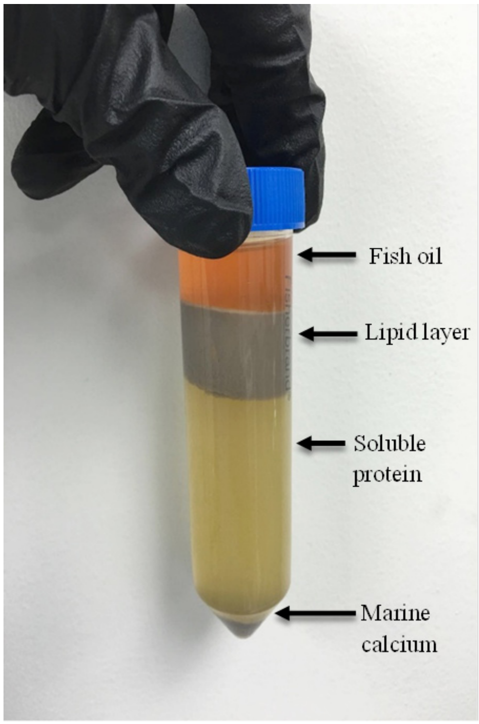

**Figure 3:** Separated three layers after enzymatic hydrolysis of salmon frames.

### Oil Analysis

Oil analysis for fatty acid composition was conducted by Jennette Wells, at Aquatic Research Cluster Facility, Memorial University of Newfoundland and this method was sent by her, through personal correspondence. This procedure was based on the method developed by Copeman, Parrish [13] and was further modified by other research groups [12-15]. For all samples of extracted oil obtained from different batches of salmon by-products received at different periods, fat/lipid extracts were esterified using sulfuric acid and methanol for 1 hour at 100°C. The Fatty Acid Methyl Esters (FAME) were analysed on a HP 6890 GC FID equipped with a 7683 autosampler. The GC column was a ZB wax+ (Phenomenex, U.S.A.) with length of 30m and internal diameter of 0.32mm. The initial column temperature was 65°C, which was held at this temperature for 0.5 minutes. The temperature was ramped to 195°C at a rate of 40°C /min, was held for 15 minutes then was further ramped to a final temperature of 220°C at a rate of 2°C /min, which was maintained at 0.75 minutes. The carrier gas was $H_2$ with flowrate of 2 mL/minute. The injector temperature started at 150°C and was ramped to a final temperature of 250°C at a rate of 120°C /minute. The detector temperature was kept constant at 260°C. 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. A quantitative standard purchased from Nu-Chek Prep, Inc (product number GLC490) was used to check the GC column about every 300 samples (or once a month) to ensure that the areas returned are as expected.

## Results and Discussion

### Chemical Characteristics of Fish By-products

There are several studies accounting the content of different components in edible parts of the Atlantic salmon [16-20]; however, there are very few studies focused on the by-products [11]. Salmon by-products can be mainly categorized as heads, viscera and frame. Recently, the possible application of various by-products has attracted much research interest for analysis of composition of fish by-products [21-23]. During this study, the different categories of by-products were analyzed separately to quantify the content of fat and protein in each fraction in future consideration to develop the value-added products from each fraction as well as to keep a record of the general characteristics of the raw material used in the present study. According to the proximate analysis (Batch A) (Table 1), viscera contain minimum amount of protein followed by frames and heads. Fat content of viscera was observed to be the highest; however, head and frames also contained substantial amount of total fat. The water activity of all the undried raw material was pretty high (~ 0.99), however moisture content was highest in heads, followed by frame and lowest in viscera. The observations of proximate analyses during current study were similar to the observations reported by Dave et al. [12].

Protein and fat content of heads and viscera were also quantified in three different fish species including Alaska pollock, Pacific cod and pink salmon, where viscera contained higher fat content and lower protein content than heads [24]. Highest salinity was observed in salmon head; however, salmon viscera fraction was observed to have very low salinity. Further, the freeze-dried sample prepared after mixing fractions of salmon frames, heads and viscera of sample received in Batch B, was analyzed for total fat and protein content (Table 2), which were equivalent to the average value of the results obtained through proximate analysis of individual parts (Table 1). From these observations, it can be deduced that salmon processing by-products are highly applicable for efficient protein and fat extraction, which can be further employed for omega-3/6/9 fatty acids preparation [25], biodiesel production [26] and preparation of protein hydrolysates [23] for supplementary applications.

### Qualitative Properties of Dried Fish By-products

Freeze-dried fish by-product mixture (Batch B) was analysed for the colour (L, a, b), water-activity and microbial content through analysis of OGYE and TPC count. The freeze-dried sample became insignificantly darker with storage both at -30 and 5°C, however the lightness factor remained constant over the storage period (Table 3). Based on the red/ green chromaticity, it was observed that the greenness of the dried sample was increased due to the initial cold temperature application, however in case of -30˚C, the redness of the sample increased with storage period. Based on the values of blue/yellow chromaticity (Table 3), all the samples were yellowish, which were neither significantly affected by storage period, nor the storage temperature. The yellowness of the sample can be due to the combined effect of the presence of high quantity of salmon oil (Table 2), which is generally reddish in color and color of other components including minerals and predominant percentage of protein in the by-product mixture (Table 2). Protein hydrolysate powders obtained through enzymatic digestion for different durations of red salmon (*Oncorhynchus nerka*) head contained 62.3% to 64.8% protein with high levels of essential amino acids were yellowish in color as observed by Sathivel et al. [27]. During an auto-oxidation study of salmon oil, at 40 days, the oil was identified as highly rancid, where the color had reportedly changed from dark red to pale yellow [28]. The oxidation of the dried sample was not analyzed during current study, however, the yellowness of the dried sample might be due to the oxidation of raw material prior to drying, leading to yellowness of the dried sample, as there was no further change in the yellowness during storage. Oxidation rate could have increased due to the mincing of the product, leading to increased exposure to oxygen. Further studies correlating the oxidation and color quality of the dried sample should be conducted which would help in prediction of quality of the material without affecting the sample.

| **Proximate analyses components** | **Salmon by-products** | | |
|---|---|---|---|
| | **Head** | **Frame** | **Viscera** |
| Protein (% of dry weight) | 40.317 ± 3.237 | 35.074 ± 3.268963 | 20.059 ± 18.89503 |
| Fat (% of dry weight) | 48.699 ± 1.237 | 52.100 ± 1.202 | 57.581 ± 6.485 |
| Moisture (% Wet basis) | 66.907 ± 2.453493 | 55.585 ± 5.684632 | 51.538 ± 3.486 |
| Water activity [Temp (˚C)] | 0.998 ± 0.002 | 0.998 ± 0.002 | 0.993 ± 0.005 |
| | [22.750 ± 0.332] | [22.825 ± 0.150] | [22.700 ± 0.100] |
| Salinity [Temp (˚C)] | 0.51 ± 0<br>[21.267 ± 0.058] | 0.488 ± 0<br>[21.1 ± 0] | 0.227 ± 0.006[23.6 ± 0] |

**Table 1:** Table summarizing the proximate analysis of the different by-products obtained from salmon processing in Batch A.

| **Sample** | **Ash content (% dry wt)** | **Protein content (% dry wt)** | **Fat content (% dry wt)** | **Moisture content (% wet basis)** | **Salinity (%)** |
|---|---|---|---|---|---|
| Freeze-dried sample | 6.481 ± 0.757 | 29.084 ± 3.979 | 56.884 ± 1.439 | 58.634 ± 0.231 | 0.303 ± 0.012 |

**Table 2:** Characteristics of freeze-dried salmon by-products mixture including head, frame and viscera (Batch B).

| **Sample** | **Days** | **Colour** | | | **Water activity** | **Temperature** |
|---|---|---|---|---|---|---|
| | | Average L ± SDV L | Average A ± SDV A | Average B ± SDV B | Mean ± SDV | Mean ± SDV |
| Freeze dried HVF | | 50.15 ± 0.97 | -0.54 ± 0.19 | 24.38 ± 1.07 | 0.053 ± 0.010 | 22.3 ± 0.1 |
| Freeze-dried HVF stored at -30˚C | 14 | 48.33 ± 1.06 | -0.86 ± 0.18 | 24.20 ± 1.09 | 0.071 ± 0.005 | 21.3 ± 0.1 |
| | 28 | 48.27 ± 1.60 | 0.29 ± 0.76 | 24.39 ± 1.52 | 0.051 ± 0.007 | 21.2 ± 0.2 |
| | 35 | 48.82 ± 2.78 | 0.49 ± 0.38 | 24.28 ± 0.77 | 0.049 ± 0.008 | 21.9 ± 0.1 |
| Freeze-dried HVF stored at 5˚C | 14 | 49.08 ± 0.48 | -0.89 ± 0.41 | 24.44 ± 1.28 | 0.065 ± 0.004 | 21.9 ± 0.2 |
| | 28 | 47.34 ± 2.38 | -1.24 ± 0.20 | 24.37 ± 0.72 | 0.052 ± 0.010 | 20.6 ± 0.3 |
| | 35 | 48.61 ± 0.71 | -0.69 ± 0.26 | 21.74 ± 1.08 | 0.062 ± 0.011 | 21.3 ± 0.2 |
| HVF: Salmon head, viscera and frame mixture | | | | | | |

**Table 3:** Summarization of color and water activity of different freeze-dried salmon by-product samples (Batch B) at different temperatures and time of storage.

| Response | Common name | Unit | Minimum | Maximum | Mean ± Std. Dev. |
|---|---|---|---|---|---|
| Oil content |  | g/ 100g | 12.8565 | 17.3373 | 15.8251 ± 2.0135 |
| C14:0 | Myristic acid | % | 1.4724 | 1.6174 | 1.5403 ± 0.0607 |
| C14:1 | Myristoleic acid | % | 0.0574 | 0.0612 | 0.0584 ± 0.0019 |
| C15:0t |  | % | 0.0247 | 0.0269 | 0.0257 ± 0.0010 |
| C15:0 α-t |  | % | 0.0049 | 0.0059 | 0.0054 ± 0.0004 |
| C15:0 | Pentadecanoic acid | % | 0.1053 | 0.1198 | 0.1103 ± 0.0067 |
| C16:0 t |  | % | 0.0088 | 0.0143 | 0.0128 ± 0.0026 |
| C16:0 | Palmitic acid | % | 12.2685 | 13.2606 | 12.8738 ± 0.4809 |
| C16:1ω11 |  | % | 0.2052 | 0.3788 | 0.3119 ± 0.0838 |
| C16:1ω9 | Hexadecenoic acid | % | 0.2401 | 0.2674 | 0.2571 ± 0.0126 |
| C16:1ω7 | Palmitoleic acid | % | 4.5127 | 4.8683 | 4.7009 ± 0.1722 |
| C16:1ω5 |  | % | 0.103 | 0.1327 | 0.1169 ± 0.0123 |
| C17:0 t |  | % | 0.0595 | 0.0687 | 0.0649 ± 0.0040 |
| C17:0 α-t |  | % | 0.1336 | 0.1525 | 0.1433 ± 0.0078 |
| C16:2ω4 | Hexadecadienoic acid | % | 0.2009 | 0.2208 | 0.2078 ± 0.0088 |
| C17:0 | Heptadecanoic acid | % | 0.1126 | 0.1366 | 0.1256 ± 0.0102 |
| C16:3ω4 |  | % | 0.1183 | 0.1381 | 0.1318 ± 0.0091 |
| C17:1 | cis-10-Heptadecenoic acid | % | 0.1576 | 0.1707 | 0.1627 ± 0.0063 |
| C16:3ω3 |  | % | 0.013 | 0.0209 | 0.0152 ± 0.0038 |
| C16:4ω3 |  | % | 0.0046 | 0.0086 | 0.0067 ± 0.0016 |
| C16:4ω1 |  | % | 0.1402 | 0.185 | 0.1565 ± 0.0200 |
| C18:0 | Stearic acid | % | 3.4739 | 3.8116 | 3.6253 ± 0.1460 |
| C18:1ω9 | Oleic acid | % | 37.6585 | 40.2358 | 39.2615 ± 1.1152 |
| C18:1ω7 | Vaccenic acid | % | 3.8086 | 4.3434 | 3.9882 ± 0.2408 |
| C18:1ω5 |  | % | 0.0946 | 0.1257 | 0.1102 ± 0.0133 |
| C18:2ω6 | Linoleic acid | % | 16.8352 | 18.0254 | 17.5090 ± 0.5025 |
| C18:2ω4 |  | % | 0.048 | 0.0628 | 0.0550 ± 0.0062 |
| C18:3ω4 | cis 8,11,14-Octadecatrienoic acid; Linolenic acid | % | 0.0735 | 0.0803 | 0.0768 ± 0.0031 |
| C18:3ω6 | γ- Linolenic acid | % | 0.3457 | 0.4771 | 0.4205 ± 0.0558 |
| C18:4ω3 | Stearidonic acid | % | 0.4339 | 0.506 | 0.4815 ± 0.0324 |
| C18:4ω1 | Alpha-parinaric acid | % | 0.0877 | 0.1079 | 0.0993 ± 0.0101 |
| C20:0 | Arachidic acid | % | 0.1266 | 0.1445 | 0.1340 ± 0.0075 |
| C20:1ω11 | Gadoleic acid; cis-9-Eicosenoic acid | % | 0.1176 | 0.1588 | 0.1345 ± 0.0195 |
| C20:1ω9 | 11-Eicosenoic acid; Gondoic acid | % | 1.6102 | 2.1065 | 1.8626 ± 0.2038 |
| C20:1ω7 | Paullinic acid | % | 0.1819 | 0.305 | 0.2437 ± 0.0545 |
| C20:2ω6 | cis-11,14-Eicosadienoic acid | % | 0.9608 | 1.0194 | 0.9918 ± 0.0242 |

| | | | | | |
|---|---|---|---|---|---|
| C20:3ω6 | cis-8,11,14-Eicosatrienoic acid; Dihomo-gamma-linolenic acid | % | 0.4932 | 0.6733 | 0.5809 ± 0.0738 |
| C20:4ω6 | Araquidonic acid | % | 0.4357 | 0.4706 | 0.4557 ± 0.0146 |
| C20:3ω3 | cis-11,14,17-Eicosatrienoic acid | % | 0.0987 | 0.122 | 0.1104 ± 0.0096 |
| C20:4ω3 | | % | 0.3495 | 0.3581 | 0.3541 ± 0.0039 |
| C20:5ω3 | EPA | % | 1.7412 | 2.1795 | 1.9420 ± 0.1965 |
| C22:0 | Behenic acid | % | 0.0389 | 0.0493 | 0.0438 ± 0.0043 |
| C22:1ω11 | Cetoleic acid | % | 0.5999 | 0.9072 | 0.7391 ± 0.1285 |
| C22:1ω9 | Erucic Acid | % | 0.3848 | 0.4813 | 0.4263 ± 0.0439 |
| C22:1ω7 | | % | 0.0341 | 0.0631 | 0.0500 ± 0.0130 |
| C21:5ω3 | | % | 0.0825 | 0.0978 | 0.0881 ± 0.0071 |
| C22:4ω6 | Adrenic acid | % | 0.1192 | 0.133 | 0.1262 ± 0.0057 |
| C22:5ω6 | | % | 0.0598 | 0.0822 | 0.0708 ± 0.0092 |
| C22:4ω3 | | % | 0 | 0.0145 | 0.0092 ± 0.0066 |
| C22:5ω3 | DPA | % | 0.7755 | 0.8941 | 0.8398 ± 0.0488 |
| C22:6ω3 | DHA | % | 1.8752 | 2.4562 | 2.1334 ± 0.2603 |
| C24:1 | Nervonic acid | % | 0.1044 | 0.151 | 0.1289 ± 0.0191 |
| ΣSat | | % | 17.598 | 19.1232 | 18.1934 ± 0.6962 |
| ΣMUFA | | % | 50.9544 | 53.4037 | 52.5022 ± 1.1100 |
| ΣPUFA | | % | 28.6369 | 29.6696 | 29.0523 ± 0.4376 |
| P/S | | | 1.5515 | 1.6443 | 1.5980 ± 0.0405 |
| Σω3 | | % | 7.9702 | 8.3552 | 8.1701 ± 0.1776 |
| DHA/EPA | | | 0.9794 | 1.4106 | 1.1092 ± 0.2021 |
| Sat, S= Saturated Fatty acids; MUFA = Mono-unsaturated Fatty Acids; PUFA, P = Poly Unsaturated Fatty Acids; DHA = Docosahexaenoic acid; EPA = Eicosapentaenoic acid; ω3= Omega-3 fatty acids | | | | | |

**Table 4:** Different responses observed and calculated during the study, with their corresponding maximum and minimum levels along with mean.

Water activity of the sample first increased with storage at both -30 and 5°C, which can be attributed to the adjustment of the sample to the environmental moisture content and subsequent moisture stabilization. According to the literature study conducted for current project, no relevant reports were found regarding the thermal and water porosity of polyethylene bags at such extremely low temperatures. Also, in case of -30°C, the water activity further decreased with increase in storage time, and in case of 5°C it first decreased and then increased, which can be attributed to the change in relative humidity of the storage chamber due to the storage and displacement of other products with different moisture content. However, based on the data it can be deduced that the moisture content of all samples remained very low in all the cases. Storage at lower temperatures have been observed to retain the quality of several biological commodities including color [29]. Also storage at low temperature can lead to further decrease in moisture content due to release of moisture from the biological matrix due to quick freezing in case of extremely low temperatures [30,31], and further drying due to the circulating air in the cooling and freezing chambers.

Reduction in moisture increases the storability of the biological products, which can be both attributed to the decreased microbial and enzymatic activity. In the present case, drying decreased both OGYE (Figure 4) and TPC (Figure 5), which further decreased with increase in storage time. Reduction in microbial count has been also observed in case of various commodities with decrease in moisture content [32,33]. The decrease in OGYE and TPC can be attributed to the unavailability of moisture content for the survival of micro-organisms in the present samples [32,33]. However, the microbial population was higher in case of salmon by-products stored at -30°C than at 5°C. Hence, further studies are required to confirm and identify the organisms which survived such low temperatures as -30°C in case of salmon by-products.

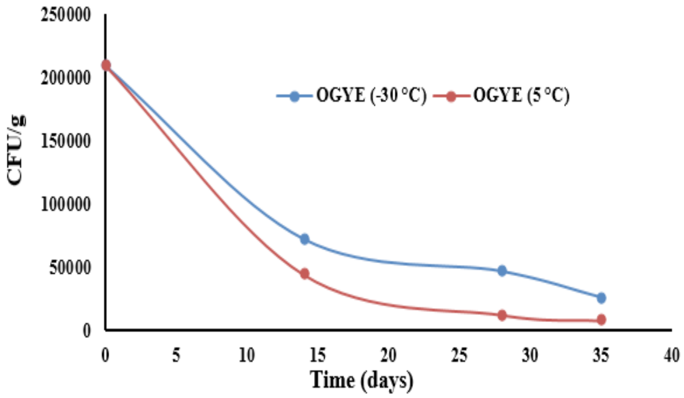

**Figure 4:** Variation of OGYE count with temperature of storage and time of storage of freeze-dried salmon by-products mix (Batch B).

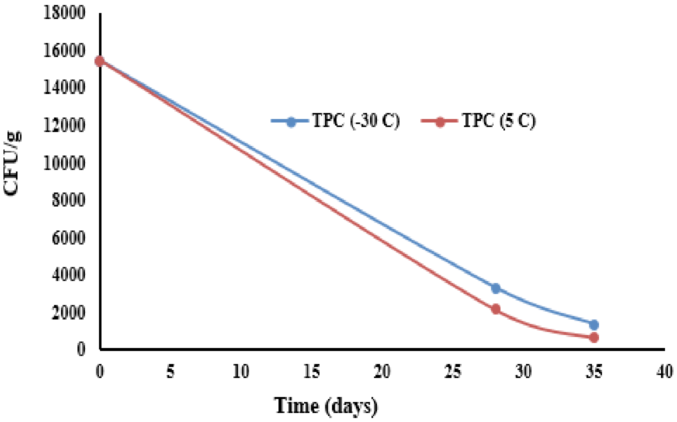

**Figure 5:** Variation of total plate count with time and temperature of storage of freeze-dried salmon by-products mix (Batch B).

## Composition of Oil Extracted from the Salmon Frames Harvested at Different Periods of a Season

Lower temperatures have been widely applied for the storage of several animal products [29,34,35]. As mentioned before, storage at -30˚C in the previous part of the study maintained the microbial and qualitative properties of the salmon by-products. Hence, to maintain the quality of salmon by-products employed for oil extraction and fatty acid analyses, salmon frames obtained in different batches were stored at -30˚C. The oil content measured in different parts of the salmon fillet have been reported between 2.37% to 18.55% [20]. In present study, the amount of oil extracted ranged between 12.85 to 17.33 g/ 100 g of oil sample, where the variation can be mainly attributed to the processing and handling methods. However, increased storage leads to increased oil extraction, which can be attributed to the disintegration of biological matrix due to longer storage period caused by microstructural changes attributed to ice crystals formation (Figure 6) [36-38]. The different fatty acids identified and quantified in salmon frame oil have been summarized in Table 4. In the present study, the major components of the fish oil extracted from the frame consisted of Mono-Unsaturated Fatty Acids (MUFAs) and Poly-Unsaturated Fatty Acids (PUFAs). Saturated fatty acids consisted 18.2 % of the total weight of salmon frame oil. We also observed that oleic acid consists major portion (39.26%) of the salmon oil produced from frames.

Oleic acid is found in both animal fats and plant oils, and has been associated with Low Density Lipid (LDL) minimization and subsequent benefits [39,40]. Linoleic acid was also observed in case of salmon frame oil, which comprised around 17.5% of total oil content. Linoleic acid also lowers LDL cholesterol [41], which has several health benefits [42-44], including effectiveness against atherosclerosis and cardio-vascular disorders [45,46]. Though salmon frame oil contained lower amount of saturated fats, significant amount of palmitic acid was also present in salmon frame oil. Salmon oil has been reported as a good source of omega-3 and omega-6 fatty acids [47]. In the present case, DHA and EPA content was around 4 % of the total mass of the oil extracted and average amount of DPA observed among all the samples was around 0.84%. Average content of omega-3 fatty acids consisted 8.2 % and average DPA/EPA ratio was observed as 1.1%. Other fatty acids whose content was more than 1 % included myristic acid (1.54%), palmitoleic acid (4.7%), stearic acid (3.6%), vacenic acid (3.9%), gadoleic acid (1.86%). Similar composition of the salmon by-products oil was observed during previous studies [12,48].

Among the samples harvested at different time periods, DHA/ EPA ratio, total omega-3 content PUFA content were not suggestively different; however, mono-unsaturated fatty acids decreased, and saturated fatty acids increased with increased storage period (Figure 6). In a study by Wu, Bechtel [11], salmon heads and viscera were stored at 6 and 15˚C for 4 days, where free- fatty acids content increased with storage; however, omega-3 fatty acids content wasn't affected. In case of individual saturated fatty acids, generally the content increased with storage period (Figure 7). However, there was no fixed pattern observed in case of Heptadecanoic acid (C17:0), Arachidic acid, Behenic acid (C22:0), Nervonic acid (C24:1, n-9) and cis-10-Heptadecenoic Acid (C17:1), whose individual concentrations in salmon frame oil, were very low (lower than 0.5 %) (Figure 8). In case of unsaturated fatty acids, there was no fixed pattern of variation of concentrations of individual fatty acids in the salmon frame oil.

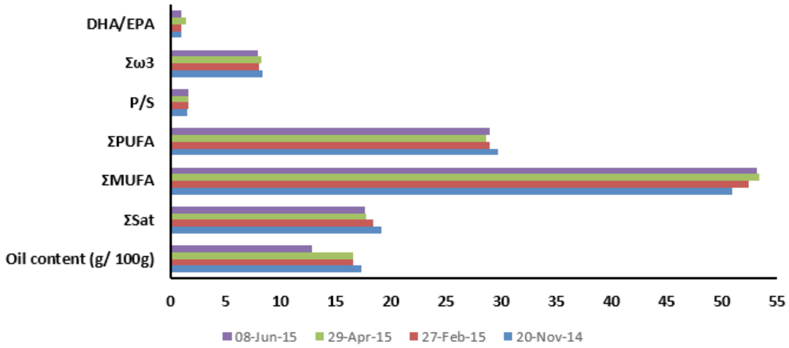

**Figure 6:** Figure presenting content of major components and ratio of major components obtained through enzymatic extraction from the fish frame samples (Batch A, B, C and D) harvested at different periods of year.

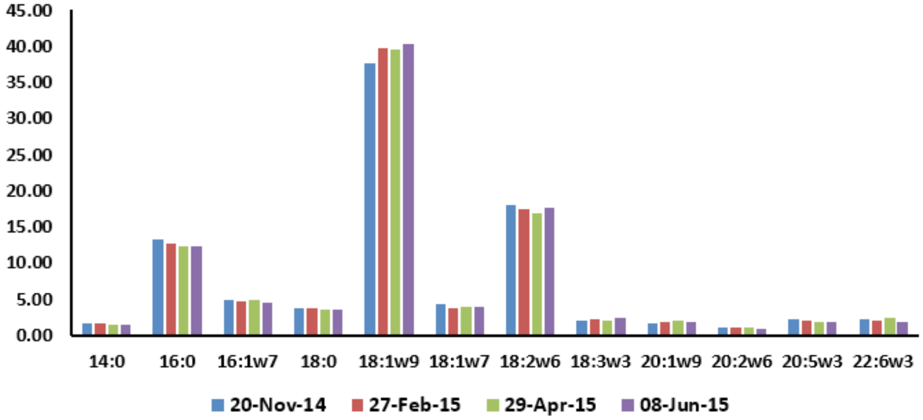

**Figure 7:** Figure presenting content of fatty acids obtained through enzymatic extraction from the fish frame samples (Batch A, B, C and D) harvested at different periods of year in major amount.

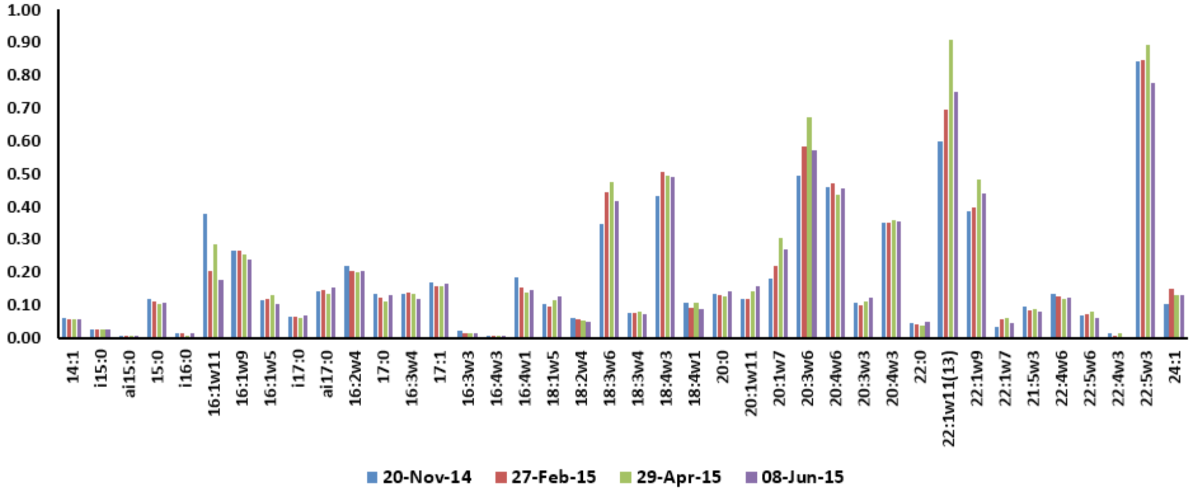

**Figure 8:** Figure presenting content of fatty acids obtained through enzymatic extraction from the fish frame samples (Batch A, B, C and D) harvested at different periods of year in minor amount.

## Conclusion

This is one of the first studies assessing the quality parameters of the dried salmon by-products. Color of the salmon by-product was not significantly affected during the low temperature storage and water activity also remained very low. Though, polyethylene transparent bags can be potentially employed as a storage bag for the packing of dried salmon-by-products for a short period of 35 days at low temperature, further studies should be conducted to confirm their effectiveness for long-term storage at very low temperatures. Microbial count decreased with drying which further depleted with increased storage time. However, TPC and OGYE count at -30 ºC were higher than observed at 5˚C. Hence, further studies are necessary to identify these microorganisms which can survive such low temperatures in salmon by-product.

In the later part of this study, salmon oil from the fish frames, harvested at different periods starting from Nov 2014 to June 2015 and stored at very low temperature (-30˚C), was enzymatically extracted. Oil yield obtained through enzymatic extraction increased with storage period, however further studies are required to optimize storage period as a conditioning factor for obtaining higher oil yield. Saturated fatty acids increased and MUFAs decreased with increase in storage period. However, PUFAs and omega-3 fatty acids were least affected. Hence, it can be concluded that, storage at very low temperatures can maintain the quality of salmon fish frames for oil extraction for its further application to concentrate omega-3 fatty acids or biodiesel production; however, further studies should be conducted to observe effect of storage at very low temperature on salmon by-products, for more than 8 months to obtain replicable conclusive results.

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