

Quality of Crude Oil Extracted from Aging Walleye Pollock (*Theragra chalcogramma*) Byproducts

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Abstract The quality of crude oil was examined as a function of the temperature and delay time between collecting and processing the fish byproducts from a commercial cannery into crude oil. Extracts from pollock byproducts stored at 6 and 15 °C for up to 4 and 10 days was examined. Longer storage of byproducts resulted in higher free fatty acid (FFA) and an increase in retinol levels in the extracted crude oils. Primary and secondary oxidation assays consisting of peroxide value and thiobarbituric acid-reactive substances increased initially with storage time on day 1 followed by an unexpected decrease with storage time. Higher storage temperature resulted in greater FFA and retinol levels, but lower oxidation products in the crude oils. The tocopherol levels decreased with storage time of the byproducts with no differences between the storage temperatures. Eicosapentaenoic acid, docosahexaenoic acid and polyunsaturated fatty acids percentage remained similar in the extracted oils with the respective storage time or temperature treatments.

Keywords Crude oil · Fat soluble vitamin · Lipid oxidation · Freshness pollock byproduct

Introduction

The nutritional benefits of incorporating fish into the diet are widely acknowledged, especially the potential health

benefits from the lipid portion of fish [1–3]. The long-chain n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been extensively studied. In addition to the high quality protein and n-3 PUFAs, fish oil contains a good source of fat soluble vitamins [4]. Pelagic fish accounts for the majority of the fish oil produced in the market [5]; however, to meet increasing demands for human and animal diets other sources are being explored. Good quality oil has been reported in extracts from byproducts in steelhead trout [6], herring [7], pink salmon [8], pollock [9] and catfish [10].

The walleye pollock (*Theragra chalcogramma*) constitutes the largest single-species commercial fishery in the world. Over the past decade annual harvest ranged from 4 to 7 million tonnes worldwide with the US catches estimated at 1.5 million tonnes [11]. This world catch could result in an estimated 2.6–4.6 million tonnes of byproduct annually if all the harvest was processed for fillet and surimi [12]. The byproducts are composed of frames, heads, skin and viscera, which often end up in landfills or dumped back into the ocean after processing the fillets. Within the waste, an estimated 14.3% lipids are contained in the viscera and 1.2% in the heads [9]. The most common practice in the commercial industry for removing the oil from fatty fish is wet reduction [13]. The fish is cooked and then separated into two fractions by pressing and centrifugation, which results in a press cake and liquid. The press liquid is further separated by centrifugation into a crude oil and stickwater (aqueous) fraction based on their partitioning coefficient. The wet reduction process is applied to byproducts; however, there can be differences in the quality of the raw material due to a number of factors including long storage periods and storage at suboptimal temperatures.

Fish oil exposed to environmental elements is very susceptible to oxidation because of its high degree of

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unsaturation [14]. In addition, fish oil is also susceptible to hydrolytic spoilage with exposure to moisture and heat [15]. The degree of oxidation varies between different species of extracted fish and lipids within fish tissues [16], which are likely due to differences in polyunsaturated fatty acids [6] and naturally occurring antioxidants within the oil [8]. The products associated with lipid oxidation are undesirable because they affect the quality and nutritional value of foods [17].

Although it is commonly acknowledged that the starting raw material is critical in producing high quality oils, little is known on how oil quality changes as a function of storage time and temperature of the raw materials. While most researchers have focus on loss of oil quality after extraction from the initial fresh raw material [18–21], few have examined the changes with storage of the starting raw material prior to the extraction of the crude oil. This study has practical implications for the fish byproduct processing industry by determining the relationship of storage time and temperature on oil quality. The objective of this study was to examine the quality of the oil extracted from aging pollock byproducts on different days of storage at two different temperatures.

Materials and Methods

Sampling and Processing

Sampling of fresh walleye pollock byproducts were obtained from a commercial fish-processing plant in Kodiak, AK. Pollock were caught on 2 March 2007. The head and viscera were mechanically removed and collected randomly from the fish-processing line. The roe had been separated from viscera samples on the processing line. Immediately after collection, head, skin, frame and viscera samples were combined and separated into two bins hygienically lined with food-grade plastic holding approximately 40 L of byproducts each. One bin was stored in a walk in cooler at 6 °C and the other inside the pilot plant at 15 °C in the Fishery Industrial Technology Center. Samples were collected in triplicate on days 0, 1, 2, 3 and 4 for the 15 °C bin and days 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 for the 6 °C bin. Three replicate samples of approximately 1 kg each were randomly removed from the bins after mixing on each sampling day.

Crude oil was extracted in the laboratory from each of the replicate raw byproduct samples in an attempt to model commercial processes used in some fish meal plants. Byproducts from the respective days (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10) and temperatures (4 and 15 °C) were ground using with a 7-mm hole size plate and approximately 600 mL were transferred into each of the three glass jars.

Samples in glass jars were placed in a water bath and brought up to 95 °C with constant shaking for 15 min. The samples were transferred to 750-mL bottles and centrifuged at 2,250 g for 15 min. The resulting product included three fractions; a solid cake (bottom layer), stickwater (aqueous middle layer) and oil (upper layer). The oil fractions for each day were separated from the other fractions and combine in an amber vial for storage in the –30 °C blast freezer. Samples were further shipped to the University of Alaska Fairbanks/USDA seafood laboratory and stored at –70 °C to avoid further oxidation until analyzed.

Free Fatty Acid Determination

Free fatty acids (FFAs) were analyzed on the extracted crude oil as described by Bernardez et al. [22]. Approximately, 50 mg of oil ($n = 3$) for the representative days and temperatures were deposited into Pyrex tubes with the addition of 3 mL of cyclohexane (Fisher Scientific, PA, USA) and 1 mL of cupric acetate–pyridine (Fisher Scientific) reagent added. Tubes were vortexed for 2 min and centrifuged for 20 min. The upper layer was read at 710 nm (640 spectrophotometer, Beckman Coulter, Fullerton, CA, USA). Quantification was based on a calibration curve constructed from oleic acid (Acrōs organics, NJ, USA) standards.

Peroxide Value

Peroxide values (PVs) were measured with the official international Dairy Federation method (1991) as reported by Shantha and Decker [23]. Approximately 100 mg of crude oil was dissolved in chloroform/methanol (7/3 v/v). Ammonium thiocyanate (Acrōs organics, NJ, USA) solution was added followed by an iron (II) solution both at 50 μ L. The mixture was then incubated at room temperature and absorbance of the sample was measured at 500 nm (640 spectrophotometer, Beckman Coulter). Quantification was based on a calibration curved constructed with iron (III) chloride.

Thiobarbituric Acid-Reactive Substances

Thiobarbituric acid-reactive substances (TBARSs) were measured in the crude oil as described by Siu and Draper [24] with modification for the oil matrix. Approximately 50 mg of oil sample and 4.45 mL Cyclohexane was vortex for 1 min and then 4.5 mL of 7.5% trichloroacetic acid/0.34% thiobarbituric acid mixture (TCA–TBA) was added and homogenized for an additional 5 min. Samples were then centrifuged at 1,578 \times g for 15 min and the TCA–TBA phase was transferred and heated in a water bath at 100 °C

for 10 min then cooled to room temperature. Absorbance was measured at 532 nm on a spectrophotometer (640 spectrophotometer, Beckman Coulter) and quantification based on standard curve constructed from bis-malonaldehyde (Sigma Aldrich, MO, USA).

Fatty Acid Profile

Approximately 5 mL of crude oil extracts from fish byproducts were shipped to the University of Missouri-Columbia Experimental Station Chemical Laboratories on ice for analysis. Fatty acids were derivatized to their correspondent methyl ester and analyzed on a gas chromatograph/flame ionization detector as previously described in [9]. The fatty acid profile is based on a percent of total fat.

Vitamin Analysis

Crude oil from fish samples were weighted ($\cong 100$ –70 mg) and dissolved in 1 mL of 2-propanol for direct analysis. Internal standards consisting of retinyl acetate (Sigma Aldrich) and tocopherol acetate (Sigma Aldrich) were added into each test tube. Analysis was similar to that reported by Heudi et al. [25] with some modifications. Samples were vortexed and transferred into 2 mL vials for injection onto an HPLC system coupled to a MS equipped with a multimode source running in the atmospheric pressure chemical ionization (APCI) mode. Separation of vitamins was made on a Zorbex C8 column (150 \times 4.6 mm, i.d. 5 μ m, Agilent Technology Inc. Palo Alto, CA, USA) at a flow rate of 1.5 mL/min with an initial mobile phase of methanol/water (83/17 v/v) held for 6 min and ramped up to 86/14 v/v and held for 11.5 min. The operating conditions for the MS were as follows: 70 fragmentor voltage, 5 L/min for the drying gas flow, 58 psig for the nebulizer pressure, 250 $^{\circ}$ C for both drying gas and vaporizer temperatures, capillary voltage set at 2,000 V and corona current set at 5.0 μ A, respectively. Selective ion monitoring was used for quantification of fat soluble vitamins and the ions monitored were as follows: m/z 269.2 for retinol and retinyl acetate, m/z 432.3 for α -tocopherol and m/z 474.3 for tocopherol acetate.

Statistical Analyses

A one-way ANOVA was used to assess statistical significance in PV, TBARS, FFA and fatty acid profile from the starting extracted crude oil. Three individual samples were obtained from a pooled oil sample for the respective days. For tests of statistical significance ($P < 0.05$) between the initial sample and the following days sampled, data were subjected to Tukey's post hoc test. Statistical tests were run on Statistica version 6.0 (StatSoft Inc., Tulsa, OK).

Results and Discussion

Free Fatty Acid Content

FFA values are routinely used in assessing oil quality. FFA concentrations of crude oils extracted from byproduct stored for different periods at two temperatures are presented in Fig. 1. A linear increase in FFA levels was observed with increasing storage time of the extracted crude oil from the aged byproduct. A higher rate of FFA concentrations was noted at a temperature of 15 $^{\circ}$ C, which suggested a higher rate of hydrolysis of the ester bonds in lipids occurring at the higher temperature and the presence of water. Head and viscera byproducts from Alaskan pollock consisted of an average approximation of 72% water [26]. The starting material had FFA concentrations in extracted crude oil at roughly 0.5%, which is slightly lower than that reported in the crude oil extracted from salmon (1%) [8] and herring byproduct (0.7%) [7]. Significant differences ($P < 0.05$) in the FFA from the starting crude oil extraction were observed on day 2 for both temperatures.

The results suggested that the formation of FFA was temperature and time dependent with the stored pollock byproducts. After day 3 at 15 $^{\circ}$ C and day 7 at 6 $^{\circ}$ C the oils contained FFA levels that were above the recommended value of <7% for food-grade fish oil [27]. However, the lower quality oils could be refined to reduce high concentrations of unwanted FFA as previously suggested [28].

Peroxide Value

Initial PV on day 0 was 8.9 ± 0.8 meq peroxides/kg crude oil, which is comparable to that reported for crude

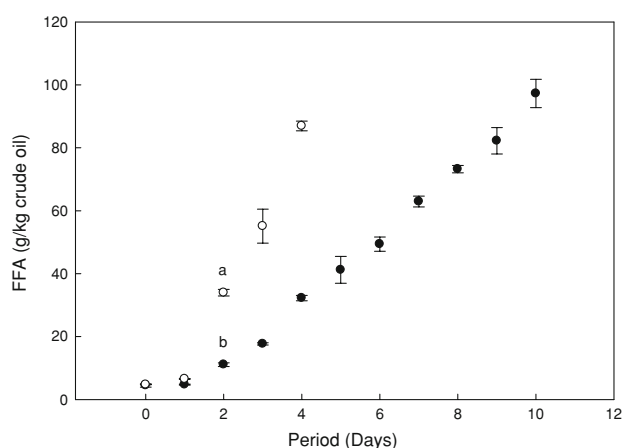


Fig. 1 Mean and standard deviation of free fatty acid in crude oils ($n = 3$) extracted from aged pollock byproduct stored at 15 $^{\circ}$ C (open circles) and 6 $^{\circ}$ C (filled circles). The letter 'a' denotes the first significant ($P < 0.05$) day from day 0 for 15 $^{\circ}$ C and the letter 'b' for 6 $^{\circ}$ C data

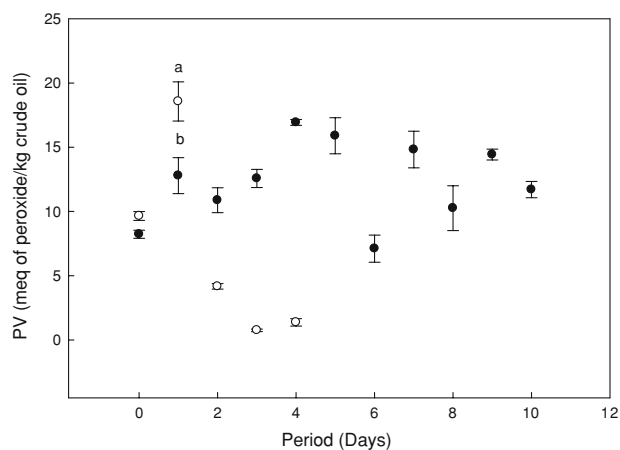


Fig. 2 Mean and standard deviation of peroxide value in crude oils ($n = 3$) extracted from aging pollock byproduct stored at 15 °C (open circles) and 6 °C (filled circles). The letter 'a' denotes the first significant ($P < 0.05$) day from day 0 for 15 °C and the letter 'b' for 6 °C data

oil extracted from mixed byproducts of herring [19]. A significant increase ($P < 0.05$) in PV was noted on day 1 at both temperatures followed by a decrease (Fig. 2). PV measures the primary oxidation products in fats and oils with the formation of hydroperoxides. The decrease in PV is possibly due to the decomposition of hydroperoxides with storage time and suggestive the rate of their degradation is much faster than the formation. During storage at both temperatures PV values showed fluctuations with maximum values at 19 and 17 meq of peroxide/kg crude oil for storage at 15 and 4 °C, respectively. Values were under 20 meq of peroxide/kg crude oil, which is generally considered necessary for oils to become rancid [29].

Thiobarbituric Acid-Reactive Substances

TBARSs measure secondary oxidation products based on the reaction of thiobarbituric acid (TBA) with malonaldehyde (MDA) and other secondary lipid peroxidation compounds. Initial starting averages of TBARS values for the two temperatures were approximately 19.3 μg of MDA eq/g of crude oil. Both temperatures of the extracted crude oil from the aged byproduct showed a significant decrease ($P < 0.5$) in TBARS from the starting material on day 6 (4 °C) and day 2 (15 °C) of the storage (Fig. 3). A faster rate of decrease was noted for the byproduct stored at 15 °C. Aidos et al. [30] also reported lower secondary oxidation products in oil extracted from herring byproduct stored at 15 and 2 °C. One explanation they postulated was that degradation increase in the raw material could result in oxidative products reacting with breakdown products (i.e. amino acids) and these complexes are being extracted out

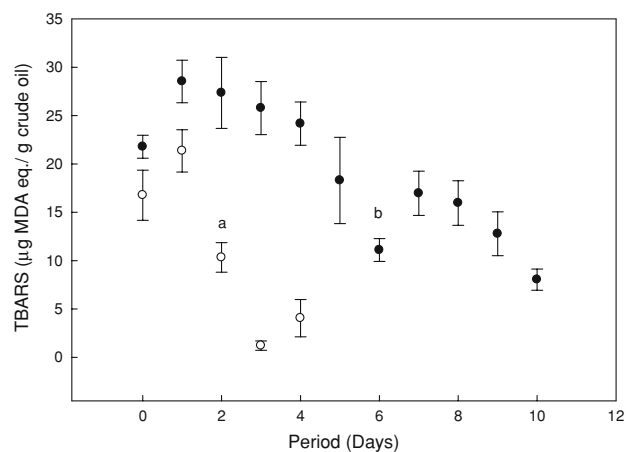


Fig. 3 Mean and standard deviation of TBARS in crude oils ($n = 3$) extracted from aging pollock byproduct stored at 15 °C (open circles) and 6 °C (filled circles). The letter 'a' denotes the first significant ($P < 0.05$) day from day 0 for 15 °C and the letter 'b' for 6 °C data

in the water phase during the processing of the oil leading to an overall decrease in the oil.

Fatty Acid Profile

Fatty acid composition of crude oil extracted from the initial byproduct and the last aged byproduct at the two temperatures are presented in Table 1. Small changes in the individual fatty acid profiles were noted from the initially extracted crude oil compared to the last aged extracted crude oil at the two respective temperatures. The largest change was an increase of 1.1% in DHA between day 0 and 4 (15 °C). The most abundant fatty acid was oleic acid (C18:1 ω 9) at approximately 17%. The second most abundant fatty acid was EPA, (C20:5 ω 3) at approximately 15% followed by palmitic acid at 9%. These results showed that pollock byproducts could be kept at the respective temperatures and storage time with no major loss of EPA, DHA and PUFAs. Other studies with longer storage times of up to 12 months have also shown little change in EPA and DHA in marine fish oil [31, 32]. The stability noted here could be related to the natural antioxidants present in the pollock oil such as carotenoids and tocopherol. Small but significant differences existed between day 0 and last day sampled for total saturated, monounsaturated, and n-3 (PUFAs) (Table 1).

Fat Soluble Vitamins

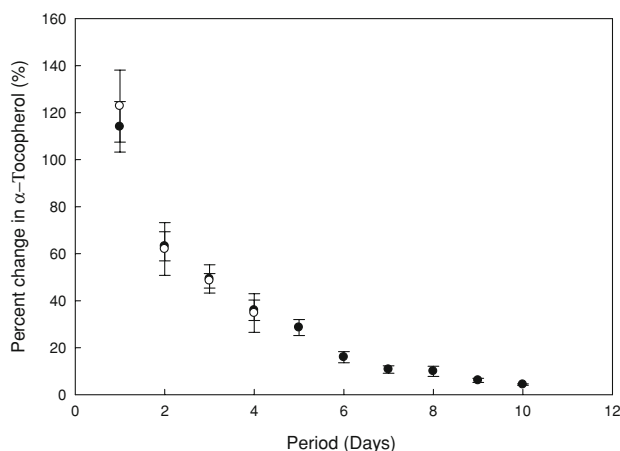
The α -tocopherol and retinol concentrations both changed in the extracted crude oil with aging of the byproduct (Figs. 4, 5). Initial concentrations of retinol and α -tocopherol were 103 and 172 $\mu\text{g}/\text{g}$ of crude oil, respectively. The

Table 1 Quantitative amounts of crude oil fatty acids extracted from pollock byproducts aged at two temperatures

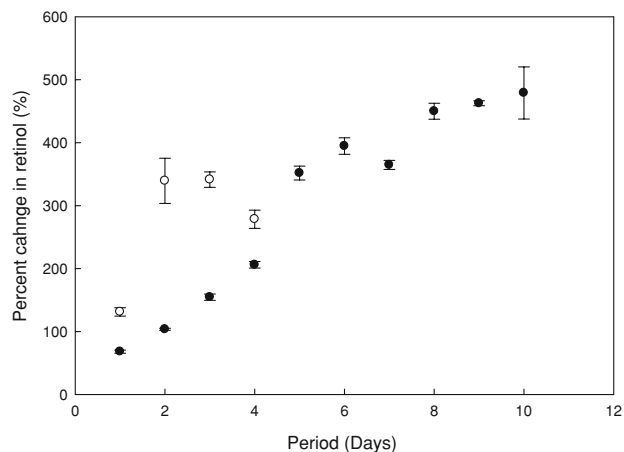
Pollock fatty acids	Total fatty acids (wt %)		
	Day 0	Day 4 (15 °C)	Day 10 (4 °C)
C14:0	4.16 ± 0.03	3.79 ± 0.01	3.94 ± 0.05
C16:0	13.54 ± 0.10	12.68 ± 0.02	13.18 ± 0.06
C18:0	2.75 ± 0.02	2.76 ± 0.01	2.85 ± 0.01
Σ Saturated	20.45 ± 0.12 ^a	19.23 ± 0.02 ^b	19.97 ± 0.08 ^c
C16:1	9.58 ± 0.04	9.31 ± 0.03	9.20 ± 0.04
C17:1	1.04 ± 0.01	1.01 ± 0.01	0.99 ± 0.01
C18:1 ω 9cis	16.82 ± 0.10	17.02 ± 0.08	16.63 ± 0.07
C18:1 ω 7cis	8.77 ± 0.13	8.90 ± 0.07	8.60 ± 0.23
C20:1 ω 9	3.41 ± 0.08	3.39 ± 0.09	3.60 ± 0.11
Σ Monounsaturated	39.62 ± 0.19 ^a	39.63 ± 0.14 ^a	39.02 ± 0.27 ^b
C18:2 ω 6	0.92 ± 0.02	0.90 ± 0.01	0.98 ± 0.01
C18:3 ω 3	3.99 ± 0.09	3.65 ± 0.05	4.05 ± 0.12
C18:4 ω 3	2.03 ± 0.01	1.84 ± 0.02	1.86 ± 0.02
C20:4 ω 6	5.20 ± 0.08	4.85 ± 0.01	5.57 ± 0.07
C20:4 ω 3	0.57 ± 0.02	0.58 ± 0.01	0.57 ± 0.01
C20:5 ω 3 EPA	15.17 ± 0.06	15.74 ± 0.05	14.90 ± 0.14
C22:5 ω 3 DPA	0.99 ± 0.02	1.10 ± 0.01	1.06 ± 0.02
C22:6 ω 3 DHA	5.55 ± 0.10	6.66 ± 0.02	6.28 ± 0.08
Σ ω 3	28.30 ± 0.15 ^a	29.57 ± 0.08 ^b	28.72 ± 0.20 ^c
Σ Polyunsaturated	34.42 ± 0.35 ^a	35.32 ± 0.16 ^b	35.27 ± 0.35 ^b

Average consisted of three crude oils. Values of 0.5% or less are not included in the Table

Rows with different superscripts (a, b, c) are significantly different ($P < 0.05$)

**Fig. 4** Changes in α -tocopherol levels in percentages from the starting crude oil ($n = 3$) extracted from aged pollock byproduct stored at 15 °C (open circles) and 6 °C (filled circles)

α -tocopherol levels decreased with storage time and the rate of depletion was similar at both temperatures. A 50% loss in α -tocopherol was noted at approximately day 3. Retinol on

**Fig. 5** Changes in retinol levels in percentages from the starting crude oil ($n = 3$) extracted from aged pollock byproduct stored at 15 °C (open circles) and 6 °C (filled circles)

the other hand increased with the aging byproduct to approximately four times the initial concentration. A higher storage temperature also resulted in a greater increase in retinol levels with time. The increased concentration with aging of raw material suggested vitamin A precursors are being converted into retinol or the unlikely possibility that retinol is derived from the spoilage organisms.

Different studies have described the conversion of carotenoids to vitamin A in different fish species [33, 34]. Byproducts in general would contain higher levels of retinol since the liver is a reservoir for vitamin A. Large variation of vitamin A levels in oil extracts from fish liver have been reported by Kinsella [35], with concentrations ranging between 10 and 50,000 μg retinol eq/liver oil. Further studies are needed to explain the mechanism involved with the increase retinol levels noted in the aged extracted crude oil.

Conclusions

The effects of storage temperature and time on oil quality from post extraction of raw pollock byproducts were examined. The results suggested that oil extracts from aged pollock byproducts at the respective temperatures and storage times exhibit good quality. An unexpected increase in the fat soluble vitamin retinol was observed in the extracted oils with increasing time and temperature in stored pollock byproducts.

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