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Salmon by-product storage and oil extraction

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ABSTRACT

Oils extracted from wild salmon by-products are excellent sources of long chain omega-3 polyunsaturated fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, quality loss is expected if time delays are encountered before oil extraction. The free fatty acid levels (FFA), fatty acid profile and total fat soluble antioxidant activity in extracted oil from aging pink salmon heads and viscera stored at two temperatures (6 and 15 °C) for four days were determined. The FFA values in raw salmon heads and viscera increased with storage time and temperature. A significant difference ($p < 0.05$) from the starting material was noted at day 1 at both temperatures for FFA. Fatty acid composition data indicated no changes in the levels of long-chain omega-3 fatty acids with the respective temperature. The concentration of long-chain omega-3 fatty acids EPA ranged from 9.3 to 11.3 g/100 g of crude oil and DHA ranged from 12.3 to 13.1 g/100 g of crude oil. The antioxidant activity of the pink salmon oils at day 0 was 0.89 ± 0.15 μmole Trolox equivalent/g of crude oil. Significant decreases ($p < 0.05$) from the starting material were noted on day 2 for 15 °C samples and day 3 for 6 °C samples. After four days of storage antioxidant levels (Trolox equivalent/g of crude oil) were approximately 25% of initial values. Oil extracted from raw salmon heads and viscera remained a good source of long chain omega-3 fatty acids even after 4 days of raw material storage at 15 °C; however, fat soluble antioxidant activity was reduced and free fatty acid levels increased with increased raw material storage temperature and time.
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1. Introduction

Marine lipids are receiving a lot of attention because of the health benefits associated with high levels of the long chain omega-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Decreased rates of cardiovascular disease have been noted in populations with high fish consumption, such as Alaskan Natives (Middaugh, 1990; Newman, Middaugh, Propst, & Roger, 1993) and the omega-3 fatty acids are believed to be associated with these health benefits (Shahidi & Miraliakbari, 2004). High levels of DHA are found in brain tissue and DHA is essential during brain development and retina formation of infants (Hoffman & Uauy, 1992). Even with the plethora of scientific evidence on beneficial effects, the consumption of omega-3 PUFA in western diet is low and alternative ways of incorporating fish oil are being explored (Nielsen, Debnath, & Jacobsen, 2007).

In Alaska there was an estimated 110,000 Mt of by-products available for production from Alaska pacific salmon in 2005 (Bechtel, 2007). The two major by-products from the processing line of salmon in Alaska are heads and viscera, with the roe removed. Wild pink and red salmon are harvested in large quantities in Alaska and pink salmon by-products have been reported to contain 10.9% oil in heads and 2% oil in viscera (Bechtel, 2003). Alaska pink salmon by-products oils were evaluated by Oliveira and Bechtel (2005) and are a good source of omega-3 fatty acid. These salmon by-products are processed into oil and meals at some location; however much of the by-products is not utilised and there are issues associated with acquiring and processing them. The ability to process salmon by-products or refrigerate large volumes of by-product are absent, especially at small remote processing plants and this problem often results in the by-products being discarded. Another problem is the during peak production by-product processing capacity can not keep up with by-product accumulation and the raw material could remain unrefrigerated for days before processing. When storage time is prolonged and temperature increased, the raw by-product quality and freshness is decreased. Degradation of by-product proceeds rapidly due to the presence of enzymes and bacteria (Ashie, Smith, & Simpson, 1996). There is no literature evaluating the storage of salmon by-products or the oil extracted from salmon by-products.

In addition to the omega-3 PUFA, fish oil also contained natural fat soluble antioxidants. While most of the focus has been on the omega-3 PUFA little information on antioxidant properties are available. Antioxidants are important because of there ability to

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scavenge free radicals, which can reduce oxidation and other damaging reactions in food and biological systems. Marine oils are highly unsaturated and very susceptible to oxidation during exposure to oxygen, light, and heat (Lytle, Lytle, Newmark, & Deschner, 1992). In addition, oxidative as well as hydrolytic stability can differ greatly among different fish species (Boran, Karacam, & Boran, 2006). Some of these differences are due to the level of protection from naturally occurring antioxidants. Vitamin E and carotenoids are antioxidants found in wild salmon oil (Johnston et al., 2006). Incorporation of synthetic antioxidant is routinely used to maintain oil quality from rendered animal products; however, the addition is accomplished after oil extraction.

There are many methods used to measure antioxidant activity including the measurement of total fat soluble antioxidant activity using a photochemiluminescence (PCL) method (Besco et al., 2007; Harrison & Were, 2007; Lee et al., 2004; Sacchetti et al., 2005). The PCL method generates photochemical superoxide anion and uses a chemiluminescent detection system in the measurement of antioxidant activity (Popov & Lewin, 1996). One of the advantages of using PCL is that it assesses the total antioxidant activities, thus synergistic effects are accounted for using this procedure.

The objective of this study was to evaluate the quality of the extracted crude oil from raw pink salmon by-products (heads and viscera) stored for 0, 1, 2, 3 and 4 days at two different temperatures (6 and 15 °C) by determining free fatty acid levels (FFA), fatty acid profile and total fat soluble antioxidant activities.

2. Materials and methods

2.1. Sampling

Fresh Alaska pink salmon (*Oncorhynchus gorbuscha*) by-products were obtained from a commercial fish-processing plant in Kodiak, AK. Salmon were caught on the 6th of August 2006 and kept on ice until processed in a commercial plant on the following day. Heads and viscera were collected randomly from the fish processing line. The roe had been separated from viscera samples on the line. Immediately after collection, head and viscera samples were combined and approximately 40 L was stored at either 6 or 15 °C in two new large (120 L) plastic trash bins, hygienically lined with food-grade polyethylene at the Fishery Industrial Technology Center (FITC) in Kodiak, AK. Temperatures were recorded twice a day and 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 for the 6 °C bin. Three replicate samples of approximately one kilogram each were randomly removed from the bins after mixing on each sampling day.

2.2. Processing

Processing of raw by-products was conducive to what would happen at a commercial feed mill plant extracting crude oil on a laboratory scale. By-products from the respective days (0, 1, 2, 3, 4) were ground using a Biro grinder model 7540 with a 7 mm hole size plate and approximately 600 ml were transferred into each of 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 using a Thermo IEC Centra – GP8 at 2250*g* for 15 min. The resulting product included three fractions; the bottom solid cake, stickwater (aqueous middle layer) and the upper oil 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 for analysis then ship to the University of Alaska Fairbanks/ USDA seafood laboratory and stored at −70 °C until analysed.

2.3. Free fatty acid determination

Free fatty acids were analysed on the extracted oil as described by Bernardez, Pastoriza, Sampedro, Herrera, and Cabo (2005). Approximately, 50 mg of oil ($n = 3$) was deposited into Pyrex tubes with the addition of 3 ml of cyclohexane and 1 ml of cupric acetate-pyridine reagent added. Tubes were vortexed for 2 min and centrifuged for 20 min. The upper layer was read at 710 nm. Quantification was based on a calibration curve constructed from oleic acid (Acrōs organics, Morris Plains, NJ) standards.

2.4. Fatty acid profile

Approximately 5 ml of crude oil extracts from fish by-products were shipped to the University of Guelph Food Laboratory on ice for analysis. Method of analysis was based on AOAC 969.33 (1990). Fatty acids were derivatized to their correspondent methyl ester. Due to the amount of crude oil needed and cost of analysis no replicate was performed for the fatty acid profile on the oils.

2.5. Antioxidant activity

A photochemiluminescence (PCL) detection method using a Photochem (Analytik Jena AG, Jena, Germany) system was used to measure antioxidant activities. The antioxidant activities were measured using the ACL kit and procedures provided by the manufacturer (Analytik Jena AG, 2005). Approximately 50 mg of extracted oil ($n = 3$) were solubilised in 1 ml of *n*-hexane (Fisher Scientific, Pittsburgh, PA) and filtered through a 0.45 μm disk filter. A portion of the solubilised oil was used to measure antioxidant activity three times. The ACL kit was used to measure lipid soluble antioxidant activity and units are reported as Trolox equivalent/g fish oil.

2.6. Statistical analysis

A one-way ANOVA and Tukey's post hoc test were used to assess statistical significance in FFA and antioxidant concentrations. Three individual samples were obtained from a pooled oil sample for the respective days. For tests of statistical significance ($p < 0.05$) between days sampled, data were subjected to Tukey's post hoc test. A *t*-test was used to evaluate significant differences between 6 and 15 °C storage temperatures for the respective day in FFA and total antioxidant concentrations. For determining any statistical significant ($p < 0.05$) between the two storage temperatures for the fatty acid composition a statistical analysis was achieved using a nonparametric method (Wilcoxon's matched pairs test) to allow for the small sample sized. Statistical tests were run on Statistica version 6.0 (StatSoft Inc., Tulsa, OK).

3. Results and discussion

The FFA values in raw salmon heads and viscera increased with storage time and temperature (Fig. 1). FFA values were used as the quality indicator of oil and a significant difference ($p < 0.05$) from the starting material was noted at day 1 for both temperatures (Fig. 1). Storage temperatures of 6 and 15 °C were chosen to reflect the colder temperatures that often prevail in Alaska on the Gulf of Alaska coast. The 15 °C samples had significantly ($p < 0.05$) higher FFA values than the 6 °C samples on days 2, 3 and 4. The levels at both temperatures began to plateau on day 2 for both storage temperatures. The maximum FFA values were 63 ± 6 g/kg of crude oil at 15 °C and 29 ± 8 g/kg of crude oil at 6 °C. The starting material had FFA concentrations of 9.6 ± 2.5 g/kg of oil or roughly 1% of total fatty acids. These FFA values are similar to what Aidos, Masbernat-Martinez, Luten, Boom, and van der Padt (2002) reported in fresh mixed herring by-products at 0.7%. The high values of FFA after four days

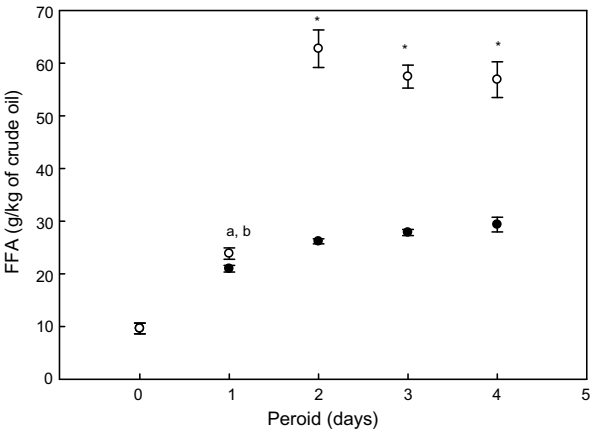

Fig. 1. Mean and standard deviation of free fatty acid in pink salmon oil ($n = 3$) extracted from salmon by-product stored at 15 °C (open circles) and 6 °C (closed circles). The letter 'a' denotes a significant difference ($p < 0.05$) for the first day from the starting material for 15 °C and the letter 'b' for 6 °C. The * represent significant difference ($p < 0.05$) between the two temperatures with in a day.

of storage are still within the recommendation of 1–7% for food grade fish oil (Bimbo, 1998). De Koning (1999) reported higher FFA in fish oil stored at 25 °C to be associated with lipolytic enzymes from microorganisms. More microorganisms would be expected with storage temperature of 15 °C than 6 °C in the raw material.

The fatty acids profiles expressed as g/100 g of crude oil extracted from the aging raw material on the respective day and temperature are shown in Table 1. Within saturated fatty acids, palmitic acid (16:0) was present in the highest concentration ranging from 13.9 to 14.8 g/100 g of crude oil. For the monosaturated fatty acids *cis*-isomer of oleic acid (C18:1ω9) was also present in substantial amounts ranging from 10.9 to 13.4 g/100 g of crude oil. Abundant PUFAs included the long-chain omgea-3 EPA (C20:5ω3), which ranged from 9.3 to 11.3 g/100 g of crude oil and docosapentaenoic acid (DPA; C22:5ω3) at 2.6 g/100 g. DHA the most abundant omgea-3 PUFA in the salmon by-product ranged from 12.3 to 13.1 g/100 g of crude oil. These values are similar to what Oliveira and Bechtel (2005) reported in fresh pink salmon head and viscera mixed together. Result of the fatty acids profile indicates that salmon oil extracted from by-products stored up to 4 days at either 6 or 15 °C had similar FAME profiles. No significant differences ($p > 0.05$) were noted in the individual FAME with the Wilcoxon's matched pairs test.

The monounsaturated fatty acids were the major fraction in the salmon by-products (Table 2) accounting for 37.4 to 40.5% of the fatty acid profile. PUFAs were the second most abundant type of fatty acid followed by SAT with values ranging from 32.7 to 35.2%. The levels of total omega 3 fatty acids were high ranging from 30.5 to 32.7%, and these values are consistent with those reported previously for salmon by-products (Oliveira & Bechtel, 2005). In this study selective destructions of fatty acids, such as the long chain PUFAs, during storage up to 4 days at was not apparent from the fatty acid profiles (Table 1) or calculations of total PUFA and omega 3 fatty acid contents (Table 2).

Total fat soluble antioxidant activity of the extracted oils during the 4 days of storage at both 6 and 15 °C decreased with time (Fig. 2). An important observation was that no significant ($p < 0.05$) difference in fat soluble antioxidant activity between the two storage temperatures was observed. Wu, Bechtel, and Bower (submitted for publication) reported a similar result for thiobarbituric acid reactive substances (TBARS) in raw pink salmon by-products and meals made from this experiment and found no significant difference between TBA values in the raw by-product for the 6 and 15 °C, storage temperatures until day 4 of storage. The mechanism is unknown that resulted in the lack of temperature effects on antioxidant activity (Fig. 2). One potential explanation is that visceral lipids are more stable against oxidation as opposed to muscle as reported by Zhong, Madhujith, Mahfouz, and Shahidi (2007) in steelhead trout.

The total fat soluble antioxidant activity of the pink salmon oils ($n = 3$) at day 0 was 0.89 ± 0.15 μmole Trolox equivalent/g of crude oil. It should be noted that the raw material is heated during the oil extraction procedure, which could reduce the antioxidant activity. Significant differences from the starting material were noted on day 2 for 15 °C and day 3 for 6 °C. On day 4, antioxidant activity at 15 °C was 0.41 ± 0.12 μmole Trolox equivalent/g of crude oil and the activity at 6 °C was 0.27 ± 0.11 μmole Trolox equivalent/g of crude oil. More variability was noted in the antioxidant activity at 15 °C, which warrants further study. The respective temperatures and storage times did not alter the fatty acid composition; however, there was a significant reduction in fat soluble antioxidant activity with approximately 25% of the initial activity remaining after 4 day of storage (Fig. 2). It should be pointed out that the mix of viscera and heads were stored and the oil extracted on days 0, 1, 2, 3 and 4. It is possible that the loss of antioxidant activity as measured using the PCL system will be different in oils extracted from separated parts (i.e. viscera and heads). Viscera and heads contain different tissues and likely a different distribution of antioxidants.

Table 1
Quantitative amounts of salmon oil fatty acids extracted from by-products stored up to 4 days at two temperatures (g/100 g of crude oil)

		Temperature 6 °C				Temperature 15 °C			
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
C14:0	5.0	4.9	5.0	4.9	4.6	4.9	4.8	5.3	4.9
C16:0	14.9	13.8	14.4	14.5	14.4	14.3	14.7	13.5	14.8
C16:1	4.8	4.5	3.9	4.5	4.6	4.6	4.4	4.7	4.5
C18:0	3.0	2.6	3.0	2.6	2.7	2.6	2.7	2.3	2.9
C18:1ω9cis	13.4	12.0	12.8	12.5	11.7	12.3	12.1	10.9	13.0
C18:1ω7cis	2.4	2.2	2.1	2.4	2.4	2.3	2.3	2.0	2.4
C18:2ω6	1.7	1.8	1.6	1.7	1.5	1.6	1.6	1.7	1.6
C18:3ω3	1.3	1.4	1.3	1.4	1.2	1.3	1.2	1.4	1.3
C18:4ω3	3.0	3.3	3.0	3.1	3.0	3.2	3.2	3.3	3.2
C20:1ω12	6.0	6.2	6.9	6.0	5.3	6.0	5.6	7.7	5.6
C20:1ω9	2.8	2.7	2.8	2.5	3.4	3.2	3.3	2.9	2.8
C20:4ω3	1.9	2.1	1.8	1.9	1.9	2.0	1.9	2.0	1.9
C20:5ω3 EPA	10.6	10.1	9.3	10.3	11.3	10.3	10.8	9.2	10.2
C22:1ω11	8.0	8.3	8.3	7.4	8.3	8.0	8.2	9.9	7.8
C22:5ω3 DPA	2.5	2.4	2.2	2.4	2.6	2.5	2.4	2.2	2.4
C22:6ω3 DHA	12.9	13.1	13.1	13.1	12.8	12.6	12.5	12.3	12.4

Values of 1% or less are not included in the table. No significant differences ($p > 0.05$) were noted between the two temperatures in the individual fatty acids with the Wilcoxon's matched pairs test for the four days.

Table 2
Summary of amounts of salmon oil fatty acids extracted from by-products stored up to 4 days at two temperatures (g/100 g of crude oil)

	Temperature 6 °C					Temperature 15 °C			
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
SAT	24.0	22.5	23.5	23.1	22.8	22.9	23.4	22.2	23.8
MUFA	39.1	38.2	38.9	37.4	37.9	38.5	38.2	40.5	38.1
PUFA	34.5	35.2	33.4	34.9	35.2	34.4	34.4	32.7	33.8
PUFA/SAT	1.4	1.6	1.4	1.5	1.5	1.5	1.5	1.5	1.4
Total omega	32.2	32.4	30.7	32.2	32.7	31.8	32.0	30.5	31.5

SAT = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids. No significant differences ($p > 0.05$) were noted between the two temperatures in the summary of fatty acids with the Wilcoxon's matched pairs test for the four days.

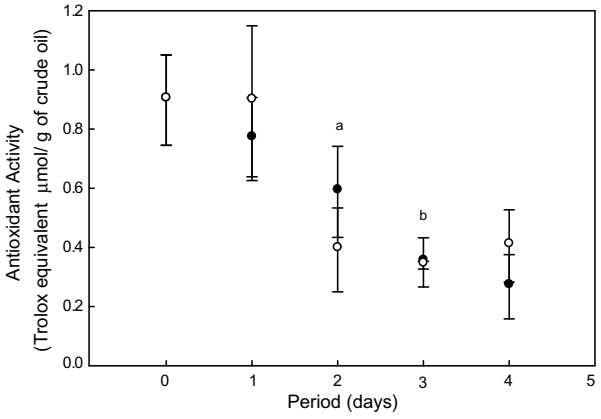

Fig. 2. Mean and standard deviation of antioxidant activity in pink salmon oil ($n = 3$) extracted from salmon by-product stored at 15 °C (open circles) and 6 °C (closed circles). The letter 'a' denotes a significant difference ($p < 0.05$) for the first day from the starting material for 15 °C and the letter 'b' for 6 °C. No significant difference ($p > 0.05$) between the two temperatures with in a day was noted.

4. Conclusions

Raw pink salmon by-product (heads and viscera) were aged at two temperatures to examine the quality of oils extracted. Although the free fatty acid content increased 3–6-fold during storage at 6 °C and 15 °C the values were in the acceptable range for many uses and the fatty acid profiles were similar. The levels of fat soluble endogenous antioxidant activity measured in Trolox equivalency units was reduced over 50%; however, substantial fat soluble antioxidant activity remained after 4 day of raw by-product storage. Results suggest that even after 4 days of storage at 15 °C, oil extracted from salmon heads and viscera is a good source of omega-3 fatty acids, especially EPA and DHA, has FFA values less than 7%, and retained approximately 25% of its antioxidant activity. This is an important study that could increase salmon by-product utilisation by showing that the oil from by-product stored for 4 days at 15 °C has a higher quality than many would anticipate. This supports the concept of salmon by-product from remote locations being transported within 4 days to a centralised processing facility for oil extraction.

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