Efficient and Economic Storage of Salmon Processing Waste as a Prospective Source for High Value Products

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Content

1. Acknowledgements

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2. Executive Summary

The following report represents the efforts of the Centre for Aquaculture and Seafood Development (CASD) research team in developing economic and efficient preservation and storage methods to preserve the quality and freshness of Atlantic salmon by-products for production of high-quality salmon oil with potential for pharmaceutical and nutraceutical use, which can lead to a growth in the regional development of Newfoundland and Labrador and enhance the competitiveness of local aquaculture industry.

Atlantic salmon aquaculture has a crucial position in Newfoundland aquaculture industry. In order to enhance its competitiveness in the global market, the efficient utilization of salmon resources is important. The salmon processing waste is abundant in lipids, which have the potential to be valorized for nutraceutical grade fish oil products. The quality of the oil product is closely related to freshness and quality of the fish materials. If the raw materials are rancid, the quality of the oil products will be inferior. Therefore, it is critical to develop efficient preservation and storage methods which can preserve the quality and freshness of the fish materials without additional high cost to the existing industrial chain. This project investigated different pretreatment methods for salmon by-products (heads, frames and viscera), including grinding, tumbling and antioxidant addition. The pretreated salmon by-products were stored at different temperatures for up to 90 days. The effect of the pretreatment methods and storage conditions were determined based on the quality of the extracted oil (peroxide value, *p*-anisidine value, free fatty acid content and fatty acid profile).

3. Glossary and Acronyms

AOX: Antioxidant

AV: p-Anisidine value

DHA: Docosahexaenoic acid

EPA: Docosahexaenoic acid

FFA: Free fatty acid

PUFA: Polyunsaturated fatty acid

PV: Peroxide value

TOTOX: Total oxidation value

4. Introduction

4.1 Project background

Newfoundland and Labrador is a province which relies its development enormously on fish and seafood industry. In 2019, the total value of fish and seafood production was close to $1.4 billion, among which 12% came from aquaculture and 88% from wild fisheries [1]. The growth of this industry leads to the rising of a series of fields, including fish feeding, aquaculture, fish harvesting, processing and relevant services for facility supplies and maintenance. During 2019, up to 15,800 workers from over 400 communities in the province were employed in this industry. However, at the same time, a large amount of processing waste from the fishery and aquaculture industry is generated every day, most of which is disposed or processed for just low value uses, such as per food or mink feed. Considering the high input in the whole industry chain, more high-value and cost-benefit products should be developed.

In Atlantic salmon processing industry, 40-50% of the fish body ends up as by-products (heads, frames and guts). Currently these by-products are mainly processed into pet food or mink feed, which are only cost-neutral or low cost-benefit. As a matter of fact, the non-fillet portion of salmon contains 15-25% lipids [2–7], and is a good source of omega-3 fatty acids that can be made into high-value pharmaceutical and nutraceutical products. There have been a variety of methods developed for the extraction of oil from salmon processing waste, among which some have been applied commercially while some are still under investigation [8–10]. The quality of the oil products can be influenced by numerous factors, among which the uppermost is the freshness of starting materials. If the raw materials are already rancid, the resulted extracted products quality would be inferior. Considering that usually the salmon by-products are not immediately processed for oil extraction after their collection from the fish processing facilities, especially in remote areas or during peak periods, therefore, it is substantial to identify the proper storage conditions, which can preserve the quality and freshness of the materials without additional high cost to the existing industrial chain.

In the present project, different pretreatment methods, including grinding, tumbling and antioxidant addition, were used to process salmon by-products (heads, frames and viscera). Different storage temperature and time were applied during the storage of the salmon by-products to investigate their impact on the quality of the oil products. This investigation not only elucidated the proper storage methods for fishery by-products as appropriate sources for further utilization, but also increased the feasibility of producing high-quality oil as potential pharmaceutical and nutraceutical products, leading

to a growth in regional development for local business and the rural communities based on aquaculture industry.

4.2 Rationale

During extraction of oil from salmon by-products, the quality of the raw materials is critical to the quality of the products. Immediately after being captured, the fish starts to spoil due to the activity of endogenous enzymes and bacteria.[11,12] The concentration of endogenous antioxidants starts to decrease, leading to oxidation of the polyunsaturated fatty acids in the lipids. Meanwhile, the amount of free fatty acids increases resulting from the ongoing hydrolysis of triglycerides in the lipids. All these procedures will accelerate the deterioration of the raw materials and thus the oil products. Specifically, for salmon by-products, very little attention has been paid for their adequate preservation compared to the fillet products, therefore they often go rancid within a short time. In order to make full use of the salmon processing waste for production of high-value products, techniques and facilities for better storage of the raw materials will be needed, which may result to extra expense to the current supply chain.

The proposed project investigated the efficient and economic pretreatment and storage methods for preservation of salmon by-products ready for extraction of premium-quality oil. The research for optimizing the production of value chain products from unutilized salmon by-products demonstrates the valid investment for aquaculture companies in the province with definite returns, and can help encourage the industry to invest more towards innovation as the whole.

4.3 Objectives

(a) Evaluation of effect of processing methods (tumbling and grinding) on the quality of the oil extracted from salmon by-products.

(b) Evaluation of effect of antioxidant addition on the quality of the oil extracted from salmon by-products.

(c) Evaluation of storage of pretreated salmon materials at different temperatures for different time.

4.4 Research methodology and approach

4.4.1 Raw materials and sample preparation

Farmed Atlantic salmon (*Salmo salar*) heads, frames, and viscera were collected in Styrofoam boxes on ice from a salmon aquaculture processing plant in Atlantic Canada, and transported to the Marine Bioprocessing Facility, Marine Institute of the Memorial University of Newfoundland, St. John's, NL. The fish materials were immediately processed once received.

4.4.2 Chemicals

Sodium thiosulfate, sodium hydroxide pellets, isooctane, glacial acetic acid, potassium iodide, sodium lauryl sulfate, and phenolphthalein (1% alcoholic) were purchased from Fischer Scientific. Ethanol (95%) was from Commercial Alcohols. Potato starch, *p*-anisidine reagent, and Alcalase 2.4L were obtained from Sigma-Aldrich. The antioxidant (rosemary extract with 4% carnosic acid and ascorbic acid) was kindly provided by Caldic Canada Inc.

4.4.3 Pretreatment of fish materials

4.4.3.1 Grinding and tumbling

Immediately after arrival, half of the fish materials were ground, and the other half was tumbled before storage. The grinding process was conducted using a meat grinder (Model 4146 The Hobart MFG. Co. Ltd.) three times with 17, 15, and 13 mm discs to obtain a homogeneous mixture. The tumbling process was performed using a tumbler (Vacuum Tumbler VTS-42, BIRO, USA) consisting of a rotatory drum and two internal baffles. Fish materials were tumbled under a vacuum of 20 mmHg for 15 min in total, including 5 min clockwise, 5 min pause, and 5 min anticlockwise of tumbling. The product obtained was an entire intact piece of the by-products loaded. The ground or tumbled materials were vacuum packaged in bags, each weighing approximately 400 g, and stored in dark at 10°C. On days 1 and 7, one batch of the samples was taken for oil extraction and analysis.

4.4.3.2 Addition of an antioxidant

10 g of the antioxidant was mixed with 500 ml of distilled water and manually stirred with a

glass spatula to form an antioxidant solution, which was added to 10 kg of the salmon by-products. In the grinding process, the antioxidant solution was added after the first cycle and manually mixed with the fish materials using a plastic spatula before proceeding to the subsequent grinding cycles. In the tumbling process, the antioxidant solution was added directly to the tumbler after loading the by-products. The drum was closed and run for 15 minutes with a vacuum of 20 mmHg. The processed salmon by-products were vacuum packaged in bags, each weighing approximately 400 g, and stored in dark at -18°C. The peroxide value, *p*-anisidine value, total oxidation value, free fatty acid content, and fatty acids profile of the oil extracted were analyzed on days 1, 30, 60, and 90 of storage.

4.4.4 Enzymatic extraction of fish oil

The frozen fish materials were thawed in cold running water and ground using a Ninja professional blender. An amount of 300 g fish sample was weighted in a 1 L Mason jar. With stirring, the pH of the fish materials was adjusted to 8 using 4 N sodium hydroxide solution (Hach HQ440d pH meter). 3 g of Alcalase 2.4U was added to initiate the reaction, and the mixture was digested in an incubator shaker (Thermo-Scientific Max Q 6000, Marietta, Ohio, USA) at 50°C and 150 rpm for 2 hours. After the reaction was completed, the mixture was heated in a water bath (Fischer Scientific Versa Bath) at 90°C for 10 minutes to deactivate the enzyme. The mixture was cooled down to room temperature and centrifuged at 6855 rpm for 10 minutes (Thermo Scientific LYNX 4000 Centrifuge). The top oil layer was collected and stored under nitrogen in the dark at -80°C until further analyses.

4.4.5 Oil quality analysis

Peroxide values (PV) were determined following the official method of American Oil Chemists' Society Cd 8b-90 [13]. *p*-Anisidine values (AV) were determined following the official method of AOCS Cd 18-90 [14]. The TOTOX value is defined as the total oxidation and calculated using the peroxide and *p*-anisidine values TOTOX value = 2PV+AV. Free fatty acid (FFA) content was determined following the official method of AOCS Ca 5A-40 [15].

4.4.6 Fatty acid profile

The analyses of the fatty acid profile were conducted by the Aquatic Research Center (ARC), Memorial University of Newfoundland.

4.4.7 Statistical analysis

All the analyses were conducted in triplicate. The data were analyzed with a one-way analysis of variance (ANOVA) at a 95% confidence level using Minitab 17.3.1. Tukey tests were performed to compare the quality of the oil obtained from different treatments.

5. Results and Discussion

5.1 Effect of processing method on the lipid quality of salmon by-products

5.1.1 Effect of processing method on salmon heads

The quality of the oil extracted from tumbled and ground heads was analyzed on days 1 and 7 of storage (Table 1).

Table 1. Quality of the oil extracted from tumbled and ground heads stored at 10°C.

Quality analysis	Ground heads		Tumbled heads	
	Day 1	Day 7	Day 1	Day 7
Peroxide value (meq/kg oil)	0.11 ± 0.05^{b}	0.67 ± 0.03^{a}	0.11 ± 0.05^{b}	0.06 ± 0.01^{b}
p-Anisidine value	1.15 ± 0.25^{a}	1.64 ± 0.28^{a}	1.37 ± 0.11^{a}	1.55 ± 0.21^{a}
TOTOX value	1.37^{b}	2.97^{a}	1.59^{b}	1.66^{b}
Free fatty acid (%)	0.63 ± 0.06^{c}	1.57 ± 0.06^{a}	0.33 ± 0.06^{d}	0.92 ± 0.1^{b}

a-d: sample means of lipid oxidation values compared by Tukey Test at 95% confidence level was performed; means in the same row that do not share the same letter are significantly different ($p < 0.05$).

The effect of high processing (grinding) was compared to that of low processing (tumbling) on the lipid oxidation of salmon heads during storage. Both of the peroxide values in tumbled and

ground salmon heads on day 1 were close to zero and not statistically different. For ground heads, the peroxide values were increased from 0.11 to 0.67 meq/kg. In comparison, the content of peroxides in tumbled heads did not increase from day 1 to day 7, suggesting no development of the primary oxidation of lipids in the heads processed using this method. Therefore, the grinding method promoted lipid oxidation, while tumbling showed no effect on lipid oxidation of salmon heads during the storage in the present study.

The *p*-anisidine values ranged from 1.15 to 1.64 in all samples without presenting a statistical difference, which suggested no development of the secondary oxidation of lipids in salmon heads during storage. This result was confirmed by the TOTOX values, which exhibited no increase in tumbled heads after 7 days of storage while showed a 2-fold increase in ground heads after the storage period. The increase in the TOTOX value of ground heads was due to the increase in the peroxide value of the same samples during storage.

As indicated in Table 1, the formation of free fatty acids was observed in all salmon head samples, indicating the activity of endogenous enzymes regardless of the processing method employed. However, the free fatty acid content in tumbled heads after storage was statistically lower than in the ground heads (0.92 and 1.57%, respectively), suggesting the prevention of lipid hydrolysis during the tumbling process. The higher content of free fatty acids in the ground heads after storage could have promoted the lipid oxidation in the samples.

Aidos et al. stored herring by-products at 15°C for 72 hr and observed fluctuating levels of peroxides and *p*-anisidine through analysis with time intervals of 24 hr [16]. The initial peroxide and *p*-anisidine values in the herring by-products were 4.4 meq peroxides/kg and 2.6, respectively, which were much higher than the values observed in salmon by-products in the present study. This is possibly due to the different oil extraction methods applied, as Aidos et al. used a scraped-surface heat exchanger while in the present study an enzymatic extraction was performed. Heat has been reported to promote oxidation during oil extraction. As reported by Dave et al. in their study about the effect of heat and enzymatic methods on the lipid oxidation of the oil extracted from salmon by-products [2], the oil extracted using the scraped-surface heat exchanger had higher total oxidation compared to the oil enzymatically extracted.

5.1.2 Effect of processing method on salmon frames

The quality of the oil extracted from tumbled and ground frames was analyzed on days 1 and 7 of storage (Table 2).

Table 2. Quality of the oil extracted from tumbled and ground frames stored at 10°C.

Quality analysis	Ground frames		Tumbled frames	
	Day 1	Day 7	Day 1	Day 7
Peroxide value (meq/kg oil)	0.42 ± 0.00^{a}	0.42 ± 0.01^{a}	0.11 ± 0.05^{b}	0.14 ± 0.00^{b}
p-Anisidine value	0.72 ± 0.24^{b}	1.29 ± 0.25^{b}	0.43 ± 0.12^{b}	0.46 ± 0.21^{a}
TOTOX value	1.56^{b}	2.12^{a}	0.65^{c}	0.74^{c}
Free fatty acid (%)	0.32 ± 0.03^{c}	1.1 ± 0.1^{a}	0.20 ± 0.0^{c}	$0.53 \quad 0.06^{b}$

a-c: sample means of lipid oxidation values compared by Tukey Test at 95% confidence level was performed; means in the same row that do not share the same letter are significantly different ($p < 0.05$).

The initial peroxide values in tumbled and ground frames had a statistical difference (0.11 and 0.42 meq/kg, respectively). However, after the storage period of 7 days, the peroxide value did not change for either of the treatments. A final peroxide value of 0.14 and 0.42 meq/kg was observed in tumbled and ground frames, respectively. A higher peroxide value developed in the ground frames compared to tumbled frames could have been promoted by the processing method. The same peroxide value on days 1 and 7 of the ground frames was possibly due to the initial increase and subsequent decrease of the content of peroxides as the lipid oxidation process proceeded during the seven days.

The *p*-anisidine values in tumbled frames were 0.43 and 0.46 on days 1 and 7, in comparison to an increase from 0.72 to 1.29 in the ground frames during the same time. Similar to peroxides, the *p*-anisidine values in the ground frames were higher than in the tumbled frames on both day 1 and 7. These results indicated the proceeding of the secondary oxidation in the oil extracted from ground frames. For this to occur, the primary oxidation should have been developed to

some extent. However, it was not observed in the peroxide analysis, probably due to the lapse between sample readings (six days).

Overall, the total oxidation value in tumbled frames slightly increased from 0.65 to 0.74 and did not show a statistical difference over time. Contrastingly, the total oxidation value in ground frames increased from 1.56 to 2.12 during storage, indicating a statistical difference between samples and between treatments.

The free fatty acid content increased with the level of processing and with time. The oil obtained from tumbled frames contained 0.2 and 0.53% free fatty acids with a statistical difference on days 1 and 7, respectively. The free fatty acids in the ground frames statistically increased from 0.32 to 1.10% during the storage. The increase of the free fatty acid content was likely due to the activity of enzymes present in salmon skin and muscle. The high free fatty acid content in ground frames could have promoted the lipid oxidation developed during the storage. Therefore, the lipid deterioration was promoted by grinding, with the increase of TOTOX value and free fatty acid content by 3 and 2 times compared to tumbled frames.

Wu et al. studied the lipid oxidation developed in minced salmon (*Salmo salar*) backbones during ice storage and reported low initial oxidation and a slight but statistically significant increase at day 11 of storage, determined by peroxide value and TBARs [17].

5.1.3 Effect of processing method on salmon viscera

The quality of the oil extracted from tumbled and ground viscera was analyzed on days 1 and 7 of storage (Table 3).

Table 3. Quality analysis of the oil extracted from tumbled and ground viscera stored at 10°C.

Quality analysis	Ground viscera		Tumbled viscera	
	Day 1	Day 7	Day 1	Day 7
Peroxide value (meq/kg oil)	0.25 ± 0.01^{c}	4.89 ± 0.46^{a}	0.08 ± 0.01^{c}	2.17 ± 0.25^{b}
p-Anisidine value	1.05 ± 0.27^{bc}	2.21 ± 0.27^{a}	0.87 ± 0.21^{c}	1.54 ± 0.11^{b}

TOTOX value	1.55[c]	12.00[a]	1.03[c]	5.88[b]
Free fatty acid (%)	4.23 ± 0.38[c]	12.35 ± 1.06[a]	1.60±0.3[d]	6.37±0.51[b]

a-d: sample means of lipid oxidation values compared by Tukey Test at 95% confidence level was performed; means in the same row that do not share the same letter are significantly different ($p < 0.05$).

The initial peroxide values in tumbled and ground viscera were close to zero, and statistically increased to 2.17 and 4.89 in tumbled and ground viscera after the storage period, respectively. The peroxide value in the oil extracted from viscera after grinding was increased by more than 2-fold after storage, resulting in significant deterioration of lipids. The *p*-anisidine values increased from 0.87 to 1.54 and from 1.05 to 2.21 in tumbled and ground viscera, respectively, indicating the progress of the secondary oxidation. There was a statistical difference between the TOTOX value of the oil from ground viscera (11.96) and tumbled viscera (5.88) after storage. Therefore, the lipid oxidation was more promoted by the grinding process in comparison to the tumbling process.

Similarly, the lipid hydrolysis in the viscera was significantly affected by the processing method. The content of free fatty acids in tumbled viscera increased from 1.60 to 6.37% during storage, while the content in ground viscera increased from 4.23 to 12.35%. The amount of free fatty acids in the ground viscera was significantly higher compared to tumbled viscera, indicating a significant promotion of the viscera lipids by the grinding process.

Wu and Bechtel determined the free fatty acid content and fatty acid profiles of the oil extracted by heat from unprocessed Alaska pink salmon (*Oncorhynchus gorbuscha*) heads and viscera [18]. The oil was stored for 4 days at 6 and 15°C and was reported with an initial free fatty acid content of 1%, which further increased to 3% and 6% at 6 and 15°C, respectively. These results are similar to those reported in the present study in tumbled viscera (1.6 to 6.37%) stored 7 days at 10°C.

5.2 Effect of antioxidant addition on lipid quality of salmon by-products

5.2.1 Effect of antioxidant addition on salmon heads

The quality of the oil extracted from salmon heads, with and without the antioxidant, was analyzed on days 1, 30, 60, and 90 of storage (Table 4).

Table 4. Quality analysis of the oil extracted from salmon heads, with and without the addition of the antioxidant, stored at -18°C (AOX: antioxidant).

Treatment	Peroxide value (meq/kg oil)			
	Day 1	Day 30	Day 60	Day 90
Tumbled heads Control	0.03 ± 0.1^{d}	0.33 ± 0.08^{bcd}	0.30 ± 0.1^{bcd}	0.31 ± 0.1^{bcd}
Tumbled heads + AOX	0.03 ± 0.05^{d}	0.36 ± 0.1^{bc}	0.25 ± 0.17^{bcd}	0.19 ± 0.1^{cd}
Ground heads + AOX	0.14 ± 0.1^{cd}	0.91 ± 0.17^{a}	0.53 ± 0.1^{b}	0.36 ± 0.1^{bc}
	p-Anisidine value			
	Day +1	Day +30	Day +60	Day +90
Tumbled heads Control	0.38 ± 0.12^{d}	1.30 ± 0.28^{ab}	1.10 ± 0.11^{bc}	0.43 ± 0.29^{d}
Tumbled heads + AOX	0.27 ± 0.01^{d}	0.20 ± 0.2^{d}	1.31 ± 0.1^{ab}	0.42 ± 0.27^{d}
Ground heads + AOX	0.55 ± 0.21^{cd}	0.74 ± 0.15^{cd}	1.76 ± 0.14^{a}	0.58 ± 0.14^{cd}
	TOTOX value			
	Day +1	Day +30	Day +60	Day +90
Tumbled heads Control	0.38 ± 0.15^{fg}	1.97 ± 0.32^{abc}	1.71 ± 0.28^{cde}	1.04 ± 0.22^{defg}
Tumbled heads + AOX	0.27 ± 0.1^{g}	0.92 ± 0.32^{efg}	1.81 ± 0.38^{bcd}	0.81 ± 0.42^{fg}
Ground heads + AOX	0.83 ± 0.35^{fg}	2.57 ± 0.22^{ab}	2.81 ± 0.31^{a}	1.30 ± 0.3^{cdef}
	Free fatty acid (%)			
	Day +1	Day +30	Day +60	Day +90
Tumbled heads Control	0.23 ± 0.03^{d}	0.42 ± 0.03^{bc}	0.45 ± 0.05^{bc}	0.52 ± 0.08^{b}
Tumbled heads + AOX	0.25 ± 0.05^{d}	0.43 ± 0.03^{bc}	0.43 ± 0.06^{bc}	0.45 ± 0.05^{bc}
Ground heads + AOX	0.38 ± 0.03^{c}	0.67 ± 0.03^{a}	0.75 ± 0.05^{a}	0.78 0.03^{a}

a-f: sample means of lipid oxidation values compared by Tukey Test at 95% confidence level was performed; means in the same row that do not share the same letter are significantly different ($p < 0.05$).

During the frozen storage study, tumbled heads (with and without antioxidant addition) and ground heads (with antioxidant added) were analyzed. The ground heads were included to analyze the effect of the grinding process. While the peroxide value exhibited a similar pattern in the tumbled heads regardless of the antioxidant addition, there was a peak of peroxide value observed for ground heads with a statistical difference on day 30. According to these results, adding an antioxidant did not affect the lipid oxidation of tumbled heads stored frozen for 90 days. In contrast, the grinding process significantly promoted the lipid oxidation of salmon heads, and this promotion effect even outweighed the suppression effect from adding the antioxidant.

The effect of the antioxidant on preventing lipid oxidation was also observed during the analysis of *p*-anisidine values. Tumbled heads without the antioxidant showed an early increase of *p*-anisidine value with a statistically significant increase on day 30, after which the value decreased, suggesting the formation of tertiary oxidation products. Tumbled and ground heads with the antioxidant had a slow development of *p*-anisidine values during the first 30 days, suggesting the effect of preventing lipid oxidation by the antioxidant. The *p*-anisidine values reached the maximum value on day 60, and decreased with further storage, which was likely due to the formation of tertiary oxidation products. Although without a statistical difference, the means of *p*-anisidine values from the ground head with antioxidant was higher than tumbled head with antioxidant.

Similarly, the total oxidation value was higher for the ground heads with antioxidant, and the TOTOX value on day 60 was significant higher compared to those from earlier days. In contrast, the tumbling heads with antioxidant showed a slow development of oxidation during the first 30 days of storage. Although a lower level of lipid oxidation was observed, these results

indicated the promotion of lipid oxidation by grinding and the prevention effect of antioxidant addition on salmon heads during frozen storage.

The free fatty acid analysis of salmon heads indicated the ongoing activity of endogenous enzymes which promoted the hydrolysis of lipids. Ground heads displayed higher free fatty acids content with a statistical difference among days 30, 60, and 90. A slower increase of free fatty acids in tumbled heads compared to ground heads confirmed the promotion effect of the grinding method on lipid deterioration of salmon heads during frozen storage. Similar to the previous study at 10˚C, the frozen study at -18˚C suggested the increased amounts of free fatty acids during storage due to the hydrolysis of lipids promoted by endogenous enzymes during the grinding process. The free fatty acids in frozen heads were formed at a slower rate than the samples stored at 10˚C, which confirmed the progression of enzyme activity under freezing conditions was slower than chilling conditions.

Wu et al. reduced the lipid oxidation in herring (*Clupea harengus*) by-products by rinsing the viscera in an antioxidant solution before mincing and storing in ice, and reported the effectiveness of antioxidant addition on the surface of by-products before storage [19]. While in the control experiment (no antioxidant) lipid oxidation of the by-products started to develop from day 1, peroxides started to form in the by-products rinsed with antioxidant on day 12, and no TBARs was observed during the entire study (12 days). In comparison, when the antioxidants were added directly in minced by-products, neither peroxides or TBARs were developed during storage, indicating higher oxidative stability of the by-products due to the mincing method compared to the rinsing/dipping method used for antioxidant addition. In this study, the grinding method was reported leading to lower oxidative stability of the lipids in the fish materials than the tumbling method. A possible explanation could be the additional process of mincing applied to the rinsed by-products, as this step could have increased the amount of oxygen in the samples, thus increasing their susceptibility to oxidation. Furthermore, the gills were excluded from salmon heads in the present study, which could have significantly reduced the hemoglobin in the system, and thereby reduced the hemoglobin-mediated lipid oxidation.

5.2.2 Effect of antioxidant addition on salmon frames

The quality of the oil extracted from salmon frames, with and without antioxidants, was analyzed on days 1, 30, 60, and 90 of storage (Table 5).

Table 5. Quality analysis of the oil extracted from salmon frames, with and without antioxidant, stored at -18°C (AOX: antioxidant).

Treatment	Peroxide value (meq/kg oil)			
	Day 1	Day +30	Day +60	Day +90
Tumbled frames Control	0.08 ± 0.08^{f}	0.64 ± 0.19^{bcd}	0.50 ± 0.14^{bcde}	0.38 ± 0.13^{cdef}
Tumbled frames + AOX	0.28 ± 0.17^{def}	0.80 ± 0.1^{b}	0.42 ± 0.0^{cedf}	0.31 ± 0.1^{def}
Ground frames + AOX	0.14 ± 0.1^{ef}	1.25 ± 0.17^{a}	0.69 ± 0.1^{bc}	0.53 ± 0.1^{bcd}
	p-Anisidine value			
	Day +1	Day +30	Day +60	Day +90
Tumbled frames Control	0.70 ± 0.05^{bc}	0.19 ± 0.15^{cde}	1.69 ± 0.13^{a}	0.28 ± 0.23^{bcde}
Tumbled frames + AOX	0.74 ± 0.2^{bcd}	0.07 ± 0.2^{de}	0.85 ± 0.48^{b}	0.04 ± 0.05^{e}
Ground frames + AOX	0.32 ± 0.25^{bcde}	0.13 ± 0.1^{e}	1.67 ± 0.12^{a}	0.30 ± 0.07^{bcde}
	TOTOX value			
	Day +1	Day +30	Day +60	Day +90
Tumbled frames Control	0.17 ± 0.19^{bc}	1.47 ± 0.27^{bc}	2.69 ± 0.28^{a}	1.05 ± 0.33^{bc}
Tumbled frames + AOX	0.56 ± 0.49^{bc}	1.67 ± 0.07^{b}	1.68 ± 0.48^{b}	0.65 ± 0.24^{c}
Ground frames + AOX	0.28 ± 0.19^{c}	2.62 ± 0.43^{a}	3.06 ± 0.07^{a}	1.36 ± 0.18^{bc}
	Free fatty acid (%)			
	Day +1	Day +30	Day +60	Day +90
Tumbled frames Control	0.30 ± 0.05^{f}	0.43 ± 0.03^{de}	0.40 ± 0.0^{def}	0.47 ± 0.06^{cd}
Tumbled frames + AOX	0.32 ± 0.03^{ef}	0.43 ± 0.06^{de}	0.47 ± 0.06^{cd}	0.48 ± 0.03^{cd}
Ground frames + AOX	0.43 ± 0.03^{de}	0.57 ± 0.03^{bc}	0.63 ± 0.06^{ab}	0.75 ± 0.0^{a}

a-f: sample means of lipid oxidation values compared by Tukey Test at 95% confidence level was performed; means in the same row that do not share the same letter are significantly different ($p < 0.05$).

The effect of antioxidant addition on tumbled and ground salmon frames was similar to salmon heads during the frozen study. The addition of an antioxidant to tumbled frames resulted in an improvement in lipid quality by preventing lipid oxidation. The grinding process facilitated the lipid oxidation of salmon frames, even with the addition of an antioxidant. The peroxide values in tumbled frames with and without the antioxidant were low and similar between these two treatments. However, the ground frames with the antioxidant contained more peroxides during storage, showing a maximum peroxide value of 1.25 meq/kg on day 30. After day 30, the amount of peroxides in the three treatments decreased, suggesting the progress to the secondary oxidation.

The second stage of lipid oxidation in frames was evident on day 60.The tumbled frames without antioxidant and ground frames with antioxidant exhibited a similar *p*-anisidine value on day 60 (1.69 and 1.67, respectively). In contrast, tumbled frames with antioxidant displayed a lower value on day 60 (0.85), suggesting the effect of the antioxidant and the tumbling method on preventing the secondary oxidation of lipids. The further decrease of the *p*-anisidine value in the three treatments on day 90 suggested the lipid oxidation likely proceeded to the tertiary stage.

The total oxidation value was higher in ground frames with the antioxidant, and was lower in tumbled frames with antioxidant. Overall, the total oxidation developed in the frozen frames during storage was low (below 3.1)..

The increase of free fatty acid content in frames followed the pattern of salmon heads and ranged around the same values. Tumbled frames with and without antioxidant showed a slightly increasing trend with a maximum of around 0.5% of free fatty acids by day 90. The free fatty acid content in ground frames with antioxidant was statistically higher on days 30, 60, and 90, with a maximum of 0.75% on day 90. The maximum value of free fatty acids reached by ground heads with antioxidant was 0.78% on the same day, indicating a similar activity of enzymes affecting the lipid hydrolysis in salmon heads and frames during frozen storage.

Wu et al. studied the lipid oxidation in mechanically deboned meat *MSM* extracted from salmon backbones with the addition of an antioxidant, and reported an inhibited lipid oxidation during

11 days of ice-storage and an absence of lipid oxidation during 8 months of storage at -20°C [19]. Similarly, in the present study, salmon frames were stable during 3 months of storage at -18°C.

5.2.3 Effect of antioxidant addition on salmon viscera

The quality of the oil extracted from salmon viscera, with and without antioxidants, was analyzed on days 1, 30, 60, and 90 of storage (Table 6).

Table 6. Quality analysis of the oil extracted from salmon viscera, with and without antioxidant, stored at -18°C (AOX: antioxidant).

Treatment	Peroxide value (meq/kg oil)			
	Day 1	Day +30	Day +60	Day +90
Tumbled viscera Control	0.08 ± 0.0^{ef}	0.42 ± 0.0^{cde}	0.42 ± 0.0^{cde}	$0.36 \pm 0.1c^{def}$
Tumbled viscera + AOX	0.06 ± 0.13^{f}	0.58 ± 0.0^{bc}	0.47 ± 0.1^{bcd}	0.33 ± 0.14^{cdef}
Ground viscera + AOX	0.22 ± 0.17^{def}	1.86 ± 0.25^{a}	0.78 ± 0.05^{b}	0.67 ± 0.08^{bc}
	p-Anisidine value			
	Day +1	Day +30	Day +60	Day +90
Tumbled viscera Control	0.45 ± 0.38^{de}	0.15 ± 0.19^{e}	1.31 ± 0.35^{b}	0.55 ± 0.3^{cde}
Tumbled viscera + AOX	0.11 ± 0.11^{e}	0.10 ± 0.07^{e}	1.02 ± 0.19^{bcd}	1.12 ± 0.4^{bcd}
Ground viscera + AOX	0.47 ± 0.19^{de}	1.23 ± 0.15^{bc}	2.30 ± 0.27^{a}	2.70 ± 0.15^{a}
	TOTOX value			
	Day +1	Day +30	Day +60	Day +90
Tumbled viscera Control	0.17 ± 0.38^{fg}	0.98 ± 0.19^{efg}	2.14 ± 0.35^{c}	1.27 ± 0.18^{def}
Tumbled viscera + AOX	0.11 ± 0.15^{g}	0.10 ± 0.07^{def}	1.02 ± 0.27^{cd}	1.12 ± 0.12^{cde}
Ground viscera + AOX	0.92 ± 0.51^{efg}	4.94 ± 0.38^{a}	3.85 ± 0.34^{b}	4.03 ± 0.29^{b}
	Free fatty acid (%)			
	Day +1	Day +30	Day +60	Day +90
Tumbled viscera Control	2.97 ± 0.67^{efg}	2.70 ± 0.26^{fg}	3.77 ± 0.15^{def}	4.13 ± 0.34^{de}
Tumbled viscera + AOX	2.93 ± 0.94^{efg}	2.43 ± 0.29^{g}	3.53 ± 0.3^{defg}	3.86 ± 0.31^{def}

Ground viscera + AOX	4.74 ± 0.39^{cd}	6.33 ± 0.12^{ab}	5.70 ± 0.09^{bc}	7.20 ± 0.53^{a}

a-f: sample means of lipid oxidation values compared by Tukey Test at 95% confidence level was performed; means in the same row that do not share the same letter are significantly different ($p < 0.05$).

In comparison to salmon heads and frames, salmon viscera exhibited a greater extent of lipid oxidation during frozen storage, which was similar as in the cold storage study. In the present study, the overall deterioration of lipids in viscera was prevented using the tumbling method, and the grinding method significantly promoted lipid oxidation.

The first stage of oxidation was slightly developed in tumbled viscera, regardless of the presence of the antioxidant. The trend on the formation of peroxides was similar and below 0.6 meq/kg during the entire storage period for tumbled viscera with and without the antioxidant. If the viscera were not ground before storage under freezing conditions, the level of lipid oxidation was as low as the heads and frames. The maximum peroxide value in ground viscera with the antioxidant was observed on day 30. After day 30, the amount of peroxides in the ground viscera with the antioxidant rapidly decreased to 0.78 and 0.67 meq/kg on days 60 and 90, respectively, suggesting the progress of the lipid oxidation to the second stage.

The analysis of *p*-anisidine value revealed the impact of the grinding process on the secondary stage of lipids. The *p*-anisidine values of tumbled viscera with and without the antioxidant increased to 1.31 on day 60 and then decreased by day 90 following the same tendency of frozen heads and frames. In contrast, the *p*-anisidine value in the ground viscera constantly increased during the entire study to 2.7 on day 90. This result indicated more extensive oxidation within the first and second stages than tumbled viscera and frozen heads and frames. It could be expected that prolonged storage of viscera over 90 days could result in more deterioration of viscera.

The TOTOX value in the ground viscera with the antioxidant was significantly higher than in tumbled viscera with and without the antioxidant. The addition of the antioxidant did not affect the total oxidation developed in tumbled viscera. However, the grinding method statistically

promoted the total oxidation of viscera on days 30, 60, and 90. A maximum TOTOX value of 4.94 observed in ground viscera on day 30 indicated a low level of total oxidation.

The high free fatty acid content is usually the limiting parameter of using salmon viscera to produce materials such as biodiesel. Stabilization of the viscera oil during storage is challenging since a variety of digestive enzymes in the viscera promoted the hydrolysis of the lipids. Heating has been widely used as an effective method to inactive enzymes. However, the heating process will promote the deterioration of other potential bio-compounds such as proteins and lipids. In the present study, the free fatty acid content in tumbled viscera with and without the antioxidant ranged from 2.43 to 4.13%. In contrast, the free fatty acid content in the ground viscera with the antioxidant increased from an initial value of 4.74% to a value of 7.2% on day 90. These results indicated the promotion of the grinding process on the lipid hydrolysis of salmon viscera.

In the present study, the added antioxidant did not affect lipid oxidation or hydrolysis of frozen salmon viscera. Yi et al. (2021) evaluated the effect of an antioxidant added to salmon (*Salmo salar*) viscera and a mix of heads and frames before oil extraction and reported that the antioxidant effectively prevented the oxidation of the extracted oils but not the lipid hydrolysis [20]. Ozen et al. (2010) studied the lipid oxidation developed in Chub mackerel (*Scomber japonicus*) mince stored at -18°C for 3 months with the addition of grape seed extract as an antioxidant [21]. A significant lower level of oxidation in the mince added with the antioxidant was observed compared with the control (no antioxidant addition). Cropotova et al. studied the lipid oxidation developed in Atlantic mackerel (*Scomber scombrus*) mince during 3 weeks of storage at -30°C and reported a peroxide value of 7.4 meq/kg of oil and TBARs of 5.4 mM/100g of sample after storage, indicating slight oxidation in the mince, which contained 12.2-13.4% of lipids (wet basis) [22]. The lipid oxidation reported in the Atlantic mackerel was higher than in the present study. The difference in oxidative stability among different fish species and parts are possibly due to many reasons, such as fatty acid profile, antioxidant/prooxidant system, season, and diet [2,17].

5.2.4 Effect of antioxidant addition on polyunsaturated fatty acid profile

The fatty acid profile analysis of the oil extracted from salmon heads, frames, and viscera, with and without the antioxidant, was performed by the Aquatic Research Center (ARC), Memorial University of Newfoundland. The content of polyunsaturated fatty acids in the oil extracted from salmon by-products with different treatments is presented in Table 7.

Table 7. Polyunsaturated fatty acid content of the oil extracted from salmon heads, frames, and viscera, with and without the antioxidant, stored at -18°C (AOX: antioxidant).

Treatment	PUFA (g/100 g oil)			
	Day 1	Day +30	Day +60	Day +90
Tumbled heads Control	29.77	29.38	29.56	29.31
Tumbled heads + AOX	29.05	30.24	29.36	29.16
Ground heads + AOX	30.03	29.52	29.31	29.58
Tumbled frames Control	28.81	29.04	28.08	28.37
Tumbled frames + AOX	28.36	27.66	28.32	28.63
Ground frames + AOX	28.00	28.39	28.65	29.55
Tumbled viscera Control	27.52	28.40	28.03	28.05
Tumbled viscera + AOX	28.23	28.34	28.37	28.17
Ground viscera + AOX	28.06	27.76	28.16	28.32

The primary polyunsaturated fatty acids (content above 1 g/100 g oil) found in the oil extracted from salmon by-products were linoleic acid (18:2ω6), alpha-linolenic acid (18:3ω3), eicosapentaenoic acid (20:5ω3), docosapentaenoic acid (22:5ω3), and docosahexaenoic acid (22:6ω3). From these, the omega-6 linoleic acid constituted approximately half of the total PUFA, with an average content of 14.5 g/kg oil, and followed by alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA), with an average content of 4, 2, 2, and 1 g/kg oil, respectively.

The content of polyunsaturated fatty acids was slightly different in salmon heads, frames, and viscera. While salmon head contained the highest content of polyunsaturated fatty acids,

viscera contained the lowest on average. The amounts of polyunsaturated fatty acids exhibited minimal changes in salmon heads, frames, and viscera during the storage period, due to the low development of lipid oxidation and a potentially strong system of endo and added antioxidants.

Dave et al. (2014) analyzed the fatty acid profile of oil extracted from salmon heads, frames, and viscera and reported approximately 39% PUFA in the oil [2]. In contrast to the present study, the content of PUFA is higher and constant among the oil from three types of by-products, and the content of DHA and EPA is significantly higher than the amount measured in the present research (2% each). In another study, Dave and Manuel reported 32.23% of PUFA, 3.48% of DHA, and 4.63% of EPA in salmon (*Salmo salar*) viscera oil. Those amounts are also above the averages found in the present study [23]. Dave and Routray characterized the oil extracted enzymatically from salmon (*Salmo salar*) frames and reported 29.05% PUFA, 2.13% DHA, and 1.94% EPA, which were similar to the results reported in the frames of the present study [24]. In another study, Routray et al. analyzed enzymatically extracted oil from salmon (*Salmo salar*) by-products (heads, frames, and viscera) and reported 29.37% PUFA, 2.56% DHA, and 2.11% EPA, indicating a low content of these fatty acids compared to previous studies [25]. The differences in fatty acid content of salmon oil were attributed to food habits and processing methods [23].

6. Recommendations for Future Research

As the effect of the antioxidant on preventing lipid deterioration was prominent in the present study, it is promising to further screen antioxidants available in the market with the aim to find one that is economically feasible as well as efficient. Moreover, further studies on refining of the oil extracted after appropriate storage and pretreatment are necessary in order to realize the valorization of fish by-products for high-value products such as nutraceuticals and pharmaceuticals.

7. Conclusions

In the present project, the effects of several different factors during the pretreatment and storage of Atlantic salmon by-products, including fish parts, processing methods and antioxidant addition were systematically investigated in terms of the oil quality and composition. The oil

extracted salmon heads and frames had lower oxidation and hydrolysis levels in comparison to viscera in both cool and frozen storage conditions. For all three types of fish parts, tumbling resulted in less deterioration of the oil compared to grinding, mainly due to less amount of air introduced during the pretreatment. The addition of an antioxidant during the pretreatment effectively promoted the quality of the oil extracted by preventing lipid oxidation, especially for tumbled materials. This is a pioneer study in the field of effective storage and pretreatment of fish by-products for the further valorization of the materials. This study will result in future support of the local industry and academic groups in the areas of by-products utilization to increase the competitiveness and sustainability for Atlantic salmon aquaculture firms.

8. References

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