OXIDATIVE DEGRADATION AND STABILIZATION OF ATLANTIC SALMON (*Salmo salar*) BY-PRODUCTS DURING STORAGE

by

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ABSTRACT

Atlantic salmon (*Salmo salar*) by-products are sources of valuable bioactive compounds such as amino acids, peptides, enzymes, collagen, and omega-3 fatty acids. Due to high perishability, leading to spoilage and loss of quality, fish by-products end up in low value products such as pet food or fertilizer. Microbial spoilage, autolysis, and lipid oxidation are the main degradative processes that occur in fatty fish. In order to optimize their use in higher value products, the degradation occurring in the salmon by-products during storage should be controlled. Adequate processing before storage is required in order to avoid the degradation of the bioactive compounds present in the by-products. The main aim of the present study was to evaluate the effect of the presence of reactive organs (which could enhance lipid oxidation) and processing methods during storage for the stabilization of Atlantic salmon (*Salmo salar*) by-products against lipid oxidation and their subsequent utilization for the extraction of high-quality bioactive compounds. The present study investigated the effect of the presence of reactive organs including gills, heart, liver, bile sac, kidney, spleen, swim bladder, and gonads. In parallel, the effect of grinding and tumbling processing methods was investigated to stabilize and increase the storage time of by-products. The lowest lipid oxidation was obtained using tumbling in the absence of reactive organs. Tumbled by-products without reactive organs were stored at -18˚C, and the protecting effect of the addition of an antioxidant against lipid oxidation of by-products using tumbling and grinding. The fatty acid profile analysis was performed on the oil extracted from frozen samples, and the oxidation of the lipids was studied. A promoting effect of lipid oxidation was observed in the ground by-products, significantly affecting viscera's lipid oxidation and hydrolysis. Salmon heads, frames, and viscera without reactive organs were stable during storage regardless of added antioxidant or the processing method (except for ground viscera). The fatty acid analysis showed five primary fatty acids present in the polyunsaturated fraction of the oils from heads, frames, and viscera: linoleic acid, alpha-linolenic acid, docosahexaenoic acid, eicosapentaenoic acid, and docosapentaenoic acid. The PUFA content remained stable during storage at -18˚C in ground and tumbled by-products with and without addition of antioxidant. However, the amounts of EPA and DHA in the present study were lower (from 3.58 to 5.7 g/100 g) compared to similar studies performed in Atlantic salmon by-products probably due to lower quantities of EPA and DHA present in the fish diet. The lipid stability of by-products containing higher levels of EPA and DHA should be investigated.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ACE	Angiotensin I- Converting Enzyme
ALA	Alpha-linolenic acid
AOCS	American oil chemists' society
AOX	Antioxidant
ARC	Aquatic Research Center
AV	*p*-Anisidine value
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl esters
FFA	Free fatty acids
FID	Flame ionization detector
FPH	Fish protein hydrolysates
GC	Gas chromatography
GmDL	Gray mullet digestive lipase
GMP	Good manufacturing practices
HHP	High hydrostatic pressure
HSPB	Hydrogen sulfide producing bacteria
HPLC	High performance liquid chromatography
LAB	Lactic acid bacteria
LDPE	Low-density polyethylene
MAP	Modified atmosphere packaging
MDA	Malonaldehyde

MSM	Mechanically separated muscle
PE	Polyethylene
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
ROS	Reactive oxygen species
Spp	Species
SSO	Specific spoilage organisms
TBARS	Thiobarbituric acid reactive substances
TBHQ	Tert-butylhydroquinone
TMA	Trimethylamine
TOTOX	Total oxidation
TVB	Total volatile bases
TVC	Total viable count
UV	Ultraviolet
VOC	Volatile organic compounds

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The work described in the present thesis was conducted by Isabel Cuenca with guidance from Dr. Deepika Dave. Isabel Cuenca was responsible for experimental design, laboratory data collection and statistical analyses. All chapters were written by Isabel Cuenca with editorial input by Dr. Jillian Westcott and Cyr Couturier. Two manuscripts from the present research are under preparation to be potentially submitted in peer review journals.

CHAPTER 1. INTRODUCTION

Atlantic salmon (*Salmo salar*) is the leading finfish species farmed globally from marine origin, representing more than 50% of the total salmon in the market; the primary producers are Norway, Chile, the United Kingdom, and Canada (Globefish, 2019). The global production of Atlantic salmon in 2018 was 2.2 million tons, with 138,000 tons produced in Canada, from which 14,000 tons were produced in Newfoundland (DFO, 2021). The market demand for products such as fillets results in the generation of up to 50% processing discards (Dave et al., 2019; Stevens et al., 2018), commonly referred to as by-products. The industry recovers value by transforming these discards into low-value products such as pet food and fertilizer; however, a significant proportion of the heads and other by-products are removed and sold fresh or fresh-frozen across the globe, particularly in Asian markets (Stevens et al., 2018). These by-products of the Atlantic salmon aquaculture industry have potential to valorize value-added products contained in the processing discards, such as protein, oil, omega 3-fatty acids, collagen, gelatin, bioactive peptides, enzymes, hydroxyapatite, and minerals (Dave et al., 2019; Gao et al., 2021; Nawaz et al., 2020; Routray et al., 2017; Terzioğlu et al., 2018).

The challenge to maximizing the value of by-products is the high perishability of these materials, resulting in the subsequent loss of their quality during storage periods. Although several reactions are involved in the degradation of fish materials, lipid oxidation is the principal reaction responsible for the quality loss of fish materials during frozen storage (Hultin, 1994; Karlsdottir et al., 2014). Lipid oxidation is a chemical reaction between oxygen and lipids in which several compounds are formed and broken down as the process evolves. The dynamic nature of this reaction requires the analysis of at least two stages of the reaction. Lipid oxidation is promoted by

internal factors such as heme compounds, metals, enzymes, and external factors such as oxygen, light, and heat (Hultin, 1994).

The level and type of processing is another factor considered to promote lipid oxidation, where processing intensity promotes higher oxidation than in low/unprocessed fish (Medina & Pazos, 2010). Processing intensity involves the size reduction of the materials and destruction of cells and tissues (e.g., mincing and grinding) (Hultin, 1994; Medina & Pazos, 2010). With low processing, the size of the material is minimally or partially reduced, and the damage to cells and tissues is lower; examples are filleting, tumbling, and immersing (Hultin, 1994; Medina & Pazos, 2010; Siró et al., 2009; Wu et al., 2020). Fish by-products contain a significant amount of heme compounds compared to other fish parts. Wu et al. (2022) reported almost seven times the hemoglobin in herring (*Clupea harengus*) by-products compared to white muscle. Fatty fish such as salmon, mackerel, and herring are particularly affected by lipid oxidation due to the high content of lipids and highly unsaturated fatty acids present in the lipids (Hultin, 1994; Medina & Pazos, 2010). Figure 1 below represents the theoretical deterioration of PUFAs undergoing lipid oxidation as a function of time (Gorkum, 2005). Hypothetically, lipid autoxidation products increase as the PUFA content decreases. However, the presence of components such as antioxidants potentially extends the time before PUFA decreases while autoxidation proceeds (Huber et al., 2009).

The quality of the value-added products can be influenced by numerous factors, among which the one of uppermost importance is the freshness of the starting materials; once the fish is slaughtered, enzymatic degradation begins almost immediately (Shumilina et al., 2016; Wu & Bechtel, 2009). If the raw materials are already rancid, the resulting quality of the extracted products would be inferior to those that are fresh. Considering salmon by-products are typically not immediately processed for value-added products extraction after their collection from fish processing facilities,

especially in remote areas or during peak periods, it is pertinent to identify the proper storage or stabilization conditions that can preserve the quality and freshness of the materials and will not bring additional costs to the process of extraction and purification of bio-compounds. Therefore, the present study aimed to evaluate storage conditions for salmon by-products to preserve their quality and subsequently increase their oxidative stability to extract premium-quality value-added products. This study represents a novel approach to increase salmon by-products oxidative stability by sorting by-products based on the inclusion or omission of reactive organs (e.g., gills, heart, liver, bile sac, kidney, spleen, swim bladder, and gonads), implementing processing methods (e.g., tumbling and grinding), and adding antioxidants (e.g., rosemary extract) during frozen storage at -18˚C.

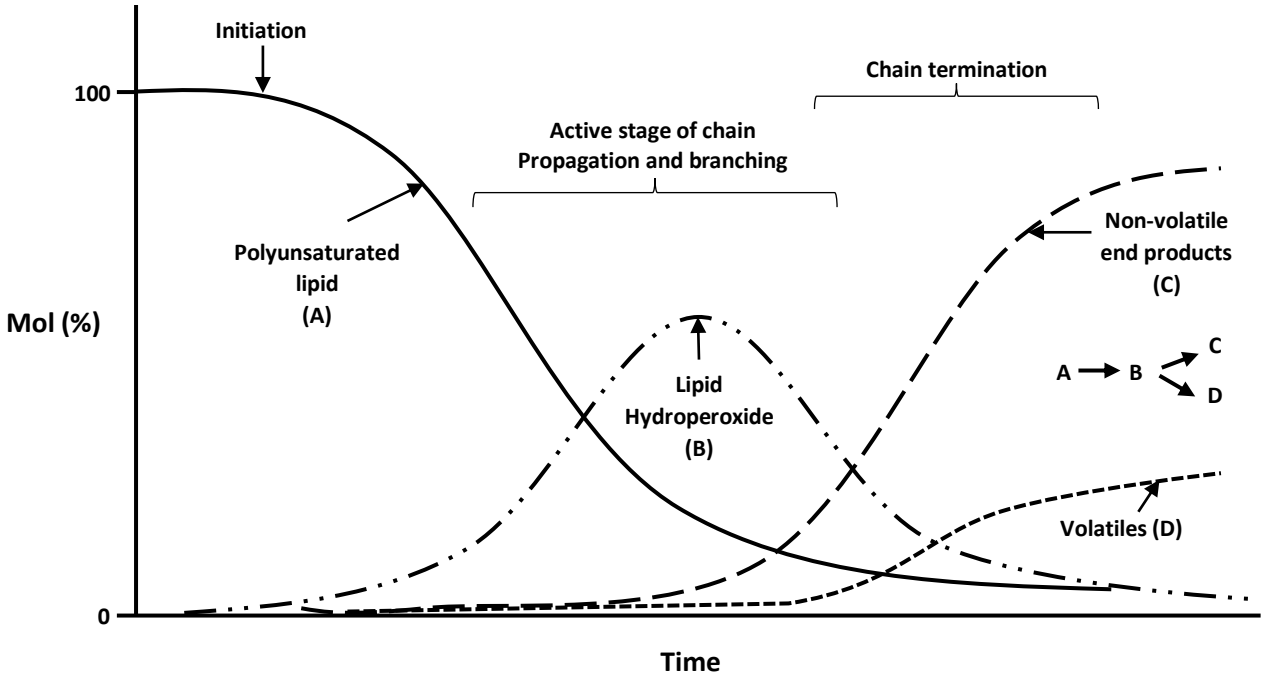


Figure 1. Time course of fatty acid (lipid) autoxidation (Gorkum, 2005).

1.1. Objectives

The main aim of the present study was to evaluate the effect of the presence of reactive organs (gills, heart, liver, bile sac, kidney, spleen, swim bladder, and gonads) and processing methods (tumbling and grinding) during storage at 10˚C, and antioxidant addition (rosemary extract) during storage at -18˚C, on lipid oxidation of Atlantic salmon (*Salmo salar*) by-products. The specific objectives were to evaluate:

1. The effect of sorting and excluding reactive organs (e.g., gills, heart, liver, bile sac, kidney, spleen, swim bladder, and gonads) from the whole by-product (all organs together) on the lipid oxidation of Atlantic salmon heads and viscera (no reactive organs were distinguished and sorted from frames) when stored at 10˚C for 7 days.
2. The effect of the processing methods of tumbling and grinding on the lipid oxidation of Atlantic salmon heads, frames, and viscera during storage at 10˚C for 7 days.
3. The oxidative degradation of salmon heads, frames, and viscera during frozen storage at -18˚C for 90 days and the effect of the addition of an antioxidant (rosemary extract), added via the processing methods tumbling and grinding, on lipid oxidation rates.

CHAPTER 2. LITERATURE REVIEW

2.1. Sustainability of salmon aquaculture

Aquaculture is the fastest growing food production system in the world, contributing substantially to the global economy. Canada ranked 22nd globally in terms of seafood production, both farmed and wild capture in 2018 (FAO, 2020). Canada is ranked 11th in the world marine and coastal aquaculture finfish production, and 16th in aquaculture mollusc production (Table 12 in FAO, 2020). In 2018, Canada produced up to 1 million tons of fish and seafood within the fisheries and aquaculture sectors. Approximately 20% came from aquaculture and 80% from fisheries landings (Figure 2) (DFO, 2021).

Salmonids, mainly including Atlantic salmon (*Salmo salar*), Chinook salmon (*Oncorhynchus tshawytscha*), and Coho salmon (*Oncorhynchus kisutch*), are the principal finfish species harvested and farmed in Canada (DFO, 2021). Atlantic salmon is the leading marine species farmed globally, being the 9th species by volume and holds 4.5% of the value share, representing more than 50% of all salmon present in the global market (Globefish, 2019). The main producing countries are Norway, Chile, the United Kingdom, and Canada. The significant markets of Atlantic salmon are represented by Japan, the EU, and North America (Globefish, 2019). With a world production of 2.2 million tons in 2018 (DFO, 2021), farmed salmon production is anticipated to grow in the coming years due to fisheries stagnation and the world's growing population (Globefish, 2019).

Canada is the 4th largest producer of salmonids globally, adding 8% to the world's production (DFO, 2012). British Columbia, New Brunswick, Nova Scotia, Ontario, Quebec, Prince Edward Island, and Newfoundland and Labrador produce salmonids in Canada (DFO, 2012, 2021).

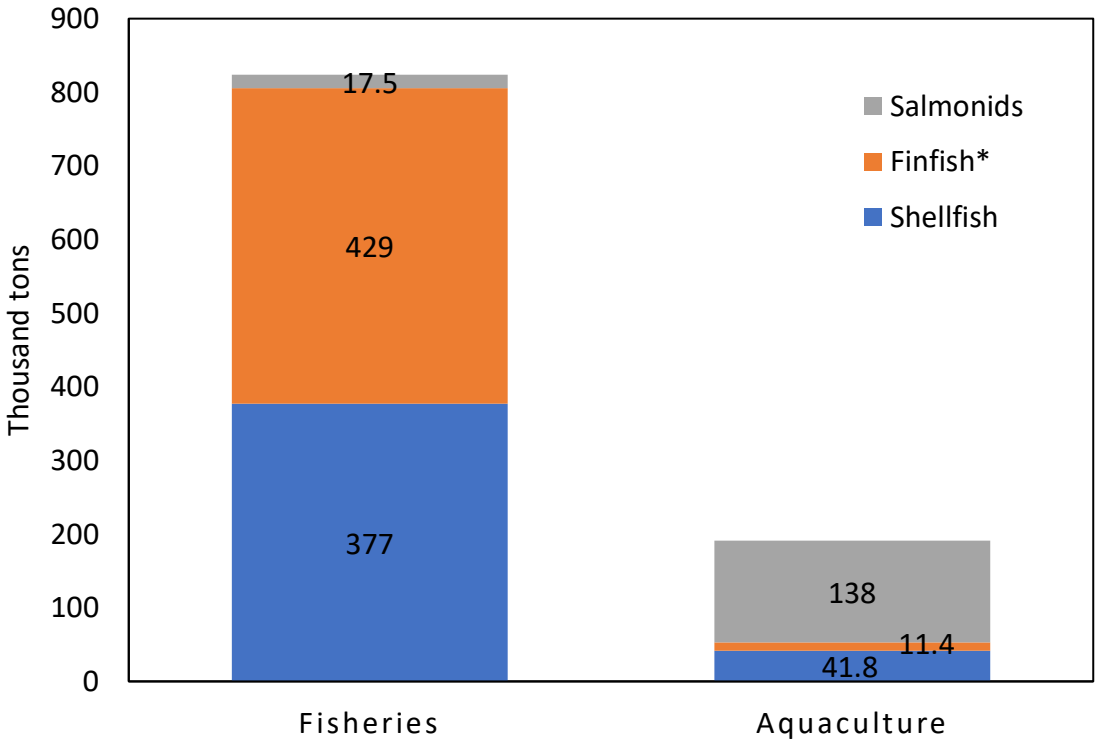


Figure 2. Fish and seafood production in Canada in 2018 (DFO, 2021).

*Finfish species excluding salmonids.

Wild captured salmonids represented approximately 12% of total salmon production in 2018 in terms of volume (DFO, 2021). In 2018, the total Canadian fish and seafood value was $5 Billion (DFO, 2021), from which $1.2 billion originated from farmed salmonids (DFO, 2021). Newfoundland and Labrador contributed 10% of total farmed salmonids in Canada in 2019 (Figure 3) (DFO, 2021).

In Newfoundland and Labrador, total finfish aquaculture production comprised salmonids (i.e., Atlantic salmon and rainbow/steelhead trout) which accounted for 15,107 tons in 2018 (DFO, 2021). This amount represented 14% of all finfish production from Fisheries and Aquaculture in the province. Interestingly, salmonid production contributed 51% of the total value generated in the province by fisheries and aquaculture (DFO, 2021). Despite the general acceptance of aquaculture's importance in meeting the global seafood demand, recurrent escapes and diseases cause socio-economic and environmental concerns (DFO, 2019; Stickney & Treece, 2012). As a result, the salmon industry focuses on strategies to reduce pollution, habitat destruction, escapes, and disease transfer (Greenberg, 2014; Healey et al., 2016). Increasing the useability of processing by-products represents one of many potential approaches toward the long-term sustainability of the industry by reducing by-products and waste (Arvanitoyannis & Kassaveti, 2008).

The market demand for salmon products such as whole-gutted, fresh, frozen, and smoked fillets has led to the generation of up to 40-50% of total biomass produced into salmon by-products, including viscera, heads, skin, scales, fins, roe, frames, trimmings, and blood (Dave et al., 2019; Stevens et al., 2018). Traditionally, farmed salmon by-products are used in low-value products such as fishmeal, silage, pet food, animal feed, fertilizer, biodiesel, and compost (Arvanitoyannis & Kassaveti, 2008; Dave et al., 2019)

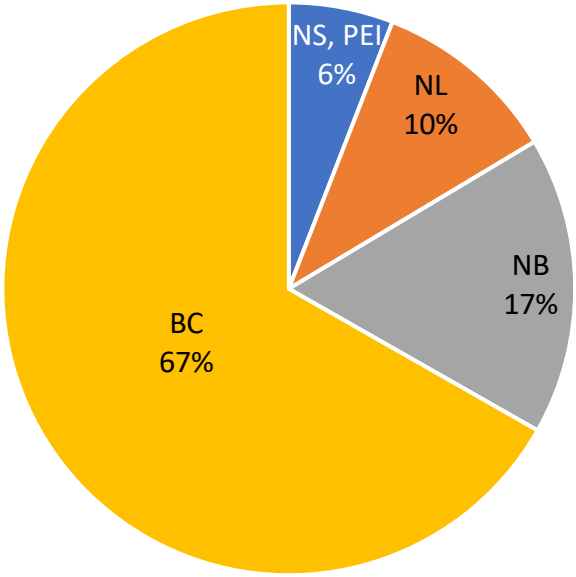


Figure 3. 2019 Production of farmed salmon in Canada by province (DFO, 2021).

BC: British Columbia, NB: New Brunswick, NL: Newfoundland and Labrador, NS: Nova Scotia, and PEI: Prince of Edward Island.

However, there is a potential to extract higher value chain products such as fish oil, omega-3 fatty acids, proteins, amino acids, bioactive peptides, fish enzymes, marine calcium, hydroxyapatite, collagen, and gelatin for use/application in the food, medical, nutraceutical, and pharmaceutical industries (Dave et al., 2019; Rustad, 2003).

2.1.1. Lower value-added products

2.1.1.1. Fishmeal

Fishmeal is the dried solid phase of a fish cake resulting from the cooking, pressing, and centrifuging fish materials (Olsen et al., 2014). Fishmeal is a common food component in fish, shellfish, pigs, poultry, and pets' diets (Suresh et al., 2018). The aquaculture sector is the principal user of this product, consuming approximately 63% of globally available fishmeal (Olsen et al., 2014; Suresh et al., 2018). Traditionally, small pelagic fish are used to produce fishmeal (Olsen et al., 2014). However, an increase in the demand for those species for human consumption necessitated an investigation into alternative sources for fishmeal production, such as fish by-products. 25% of the total fishmeal production is originated from salmon trimmings (Ytrestøyl et al., 2015), which are cut-offs from salmon fillets similar to heads, frames, viscera, fins, and skin (Kim & Mendis, 2006). The principal countries converting seafood discards into fishmeal are Canada, Japan, Chile, Denmark, Iceland, Mexico, Norway, Thailand, Russia, and the USA (Suresh et al., 2018).

2.1.1.2. Fish silage

Fish silage is a low-cost organic liquid resulting from the acidification of fish waste materials and the activity of endogenous enzymes. Fish silage is produced with organic and inorganic acids

ranging from 3.5-4.5 of pH to promote autolysis and, at the same time, to avoid spoilage (van 't Land et al., 2017). Fish silage is a good source of nutrients such as proteins, amino acids, and oils and is mainly utilized in animal feeds. The sources for fish silage production include spoiled fish, underutilized species, and seafood discards (Suresh et al., 2018). The final composition of the fish silage largely depends on the initial quality of the fish material utilized (van 't Land et al., 2017). Over 60 companies in Norway process fish rest materials, primarily producing fish silage from fish by-products (Ahuja et al., 2020; Rustad, 2003). The transformation of viscera-containing fish materials into fish silage is a convenient means to avoid the fast spoilage occurring in viscera (Olsen et al., 2014).

2.1.1.3. Fermented by-products: Sauce, flavoring compounds

Fish sauce is a liquid obtained from the hydrolysis of fish proteins through a fermentative process at high salt concentrations, whereby the decomposition of proteins is promoted by microbial and fish proteases (Do Quynh Nguyen et al., 2021). The resulting product is rich in proteins and amino acids and is generally included as a condiment with high nutritive value in Asia and other countries worldwide (Suresh et al., 2018). Meals such as *rakeorret* and *maatjes* in Europe are based on fermented fish, while *salanga* and *koobi* are examples in Africa (Marti-Quijal et al., 2020). Fish sauce is produced from species such as sardine, anchovies, round scad, and mackerel (Marti-Quijal et al., 2020; Suresh et al., 2018). Fish sauce is popularly produced from anchovy, which is among the largest fish caught in the world of low-economic value (Zhu et al., 2021). Traditionally, the fermentation process is developed by fish native bacteria, resulting in variable quality. The use of cultures such as *Marinococcus halotolerans* provides higher homogeneity in the final product (Do Quynh Nguyen et al., 2021).

Flavoring compounds are extracted from fermented fish materials and used as umami and flavor components in sauces, chowders, soups, bisques, noodles, snacks, and surimi, shrimp, and crab analogs. Seafood discards of species such as anchovies, salmon, cod, tuna, clams, crab, squid, scallops, shrimp, krill, oyster, and lobster are potential sources of flavor compounds (Suresh et al., 2018).

2.1.1.4. Biodiesel

Biodiesel is an organic alternative to petroleum diesel obtained from plant, marine, and animal fats and oils with reduced harmful air emissions (Ching-Velasquez et al., 2020; Mohiddin et al., 2021). There is an increasing interest in biodiesel production from low-cost biomass such as fish by-products (Dave & Manuel, 2014). The oil is extracted from the biomass by mechanical, solvent, and enzymatic methods (Mohiddin et al., 2021). The mechanical press is the most used method; however, it generates lower oil yields than the other extraction methods (Mohiddin et al., 2021). The solvent method such as supercritical ethanol and supercritical methanol generates higher oil yields. However, this method generates environmental concerns due to the requirement of the subsequent disposal of the hazardous chemicals used (Mohiddin et al., 2021). Environmentally friendly lipases are the most widespread enzymes used in the enzymatic extraction of biodiesel (Ching-Velasquez et al., 2020; Mohiddin et al., 2021); however, enzymatic hydrolysis requires longer processing times and higher costs than mechanical solvent methods (Ching-Velasquez et al., 2020; Mohiddin et al., 2021). Biodiesel production is affected by a high content of free fatty acids in the oil (frequently found in low-cost biomass) due to soap formation from free fatty acids and base catalysts used during biodiesel production (Mohiddin et al., 2021). In such cases, acid catalysts such as H_2SO_4, HCl, and H_3PO_4 are commonly used to perform an esterification followed by Methanol and NaOH for the transesterification of high free fatty acids feedstock (Ahmed &

Huddersman, 2022). Biodiesel has been produced from a variety of biomass sources. For example, Nguyen et al. (2018) produced biodiesel from black soldier fly larvae oil with Novozym 435 and methyl acetate. They obtained 96.97% biodiesel yield and up to 20 reaction cycles reusing the enzyme without losing enzyme activity (95.97% biodiesel yield was obtained in the 20th cycle). Costa et al. (2020) produced high-quality biodiesel from waste cooking soybean oil by hydro-esterification using ethanol and *Pseudomonas fluorescens* lipase and reported no traces of glycerol compounds in biodiesel even after recycling the enzyme six times. Ching-Velasquez et al. (2020) obtained diesel from fish waste oil using *Thermomyces lanuginosus* lipase and octadecyl methacrylate and reported a yield of 75% with an acid value of 0.9 mg KOH/g which was higher than biodiesel standards. Generally, the enzymatic methods for biodiesel production are considered expensive at an industrial scale; however, different approaches such as micro-emulsions and transesterification are mainly used methods for the biodiesel production from seafood discards (Suresh et al., 2018).

2.1.1.5. Fertilizer

The fertilizer production from fish waste represents an opportunity for the fisheries and fish farming industries to recover value from fish discards/by-products. Fish waste materials, especially bones and scales, are significant sources of Calcium, Phosphorus, and Nitrogen for meeting the demand of crops plants (Ahuja et al., 2020). The production process includes grinding, cooking, centrifugation, pressing, separating liquid and solid phases, and drying to produce liquid or dry fertilizers. Depending on the process followed, fish emulsion, fish silage, and fish compost can be obtained (Ahuja et al., 2020). More than 150 types of fish fertilizers are available in the market, and many studies indicate the effectiveness of these fertilizers in plant growth, especially in horticultural plants (Ahuja et al., 2020). Gao et al. (2021) produced liquid organic fertilizer from

fish waste utilizing thermal hydrolysis. They reported effective solubilization of fish wastes and efficient nutrient release. The bones of *Sardinella aurita* can also be used to produce calcium and phosphate-based fertilizers (Carella et al., 2021). The potential to produce fertilizer from farmed Atlantic salmon bones represents an opportunity for Canadian salmon producers.

2.1.2. Higher value-added products

The research of functional compounds present in salmon by-products has increased interest in their application in nutraceutical and pharmaceutical products (Stevens et al., 2018). Salmon by-products such as heads, frames (bones and flesh remaining in the bones), viscera, skin and scales, and trimmings are a potential source of bio compounds such as marine oils, omega-3 fatty acids, protein hydrolysates, bioactive peptides, collagen, enzymes, enzymes inhibitors and minerals, with potential application in food, medicine, and pharmaceutics (Dave et al., 2014; Liu et al., 2021; Routray et al., 2017).

2.1.2.1. Marine oils

Fish oil containing long-chain PUFAs, consisting mainly of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), provides fish with the ability to live in cold waters (Wanasundara & Shahidi, 1997). There are many benefits attributed to these oils, which are known to play a role in the nervous system, cognitive and brain development, visual development, cell signaling cascades, gene expression, cardiovascular health, thrombosis prevention, and modulates inflammatory diseases (Echeverría et al., 2017; Prabhu et al., 2021; Roke, 2017; Schwarzenberg & Georgieff, n.d.; Sugasini et al., 2017). The role of highly polyunsaturated fatty acids such as EPA and DHA, and their positive effect on diseases such as Alzheimer's, Parkinson's, schizophrenia, bipolar disorder, deep depression, autism spectrum disorders, and aging, is under investigation (Echeverría

et al., 2017; Lacombe et al., 2018; Patrick, 2019; Sugasini et al., 2017; Sun et al., 2018). The α-linolenic acid (ALA) is an essential fatty acid required for average human growth, converted into EPA and DHA in the body (Echeverría et al., 2017; Sun et al., 2018). It is found in terrestrial sources such as flaxseeds and walnuts. However, the low conversion rate of ALA in the body requires the inclusion of these fatty acids in the diet; they are essential (Sun et al., 2018). Marine oils are the only significant source of these compounds, synthesized in phytoplankton and transferred to fish and marine mammals through the food web (Globefish, 2019). The primary source of marine oil rich in omega-3 fatty acids is fish such as menhaden, sardines, and mackerel, for their low content of contaminants compared to more prominent species (Melgosa et al., 2021). Algae, seaweed, genetically engineered bacteria, and yeasts are vegan alternative sources of marine omega-3 fatty acids, although they contain lower oil than fish (Ismail, 2010; Melgosa et al., 2021). Marine oils are now produced using bioreactors with bacteria and phytoplankton and genetically engineered crops (e.g., camelina) as substitutes for the traditional methods of marine oils production (Osmond et al., 2021).

Traditionally, the oil extraction method is wet press, which uses a high temperature. Alternative methods include enzymatic, ultrasound-assisted, microwave-assisted, and supercritical fluid extraction methods. The oil yield recovered depends on the fish species and extraction method, ranging from 20% to more than 50% (dry basis) (Melgosa et al., 2021). After the crude oil recovery, a refining process is usually applied to remove unwanted compounds such as non-triacylglycerols, colorants, and toxic compounds, comprising the steps of degumming, neutralization, bleaching, and deodorization. The use of chemicals and high temperatures in the refining process results in lipid loss, PUFA degradation, and the generation of undesirable

compounds. Alternative refining methods are supercritical fluid, enzymatic and membrane processes (Rubio-Rodríguez et al., 2010).

Cold-water fish remains to be the primary source of marine oils (Barta et al., 2021), followed by microalgae and krill, which emerged as marine oil alternative sources (Barta et al., 2021; Yu et al., 2020) . However, fish by-products are also potential source for the production of edible marine oil. Aidos et al. (2003) reported 12.5% of total lipids containing 177g of PUFA, from which 66g corresponded to DHA, and 49g to EPA/Kg of lipids in fresh herring (*Clupea harengus*) by-products, which consisted of a mix of heads, frames, skin, and viscera. Wu and Bechtel (2009) characterized the oil extracted from fresh Walleye Pollock (*Theragra chalcogramma*) by-products (head, skin, frames, and viscera). They reported 34% of PUFA, of which 5.55% corresponded to DHA and 15.17% to EPA. Wu and Bechtel (2008) also analyzed the chemical characteristics of oil extracted from fresh Alaska pink salmon (*Oncorhynchus gorbuscha*) by-products, consisting of a mix of heads and viscera. They reported 32.2% of PUFA, of which 12.9% corresponded to DHA and 10.6% to EPA. Dave and Manuel (2014) evaluated the chemical properties of farmed Atlantic salmon viscera oil, reporting 32.23% PUFA, from which 3.48% corresponded to DHA and 4.63% to EPA. Routray et al. (2017) investigated the chemical characteristics of farmed Atlantic salmon (*Salmo salar*) by-products (mix of heads, frames, and viscera) oil obtained using partial drying at two temperatures (60 and 100˚C) and enzymatic extraction. The average amount of PUFA in all the experiments was 29%, of which 2.5% corresponded to DHA and approximately 1% to EPA. Dave et al. (2014) analyzed the chemical characteristics of oil extracted from farmed Atlantic salmon (*Salmo salar*) heads, frames, and viscera by enzymatic and heat extraction methods, reporting 39.74-39.83% PUFA in all by-products and extraction methods, from which 8.25-8.9% corresponded to DHA, and 7.7-8.88% to EPA.

2.1.2.2. Protein hydrolysates

Fish protein hydrolysates (FPH) are edible proteins consisting of short peptides formed from 2-20 amino acids with biological activity and functional properties (Gao et al., 2021). The biological activity of FPH is related to the prevention of human diseases such as cancer, cardiovascular, and inflammatory diseases. FPH presents functional properties suitable for their application in the food industry, such as antioxidant activity and texture functional properties such as gelling, whipping, and emulsifying (Nakkarike et al., 2010; Shahidi & Ambigaipalan, 2015). FPH is commonly produced through chemical, enzymatic, or fermentative methods. The microbial process for fermentation is regarded as safe and environmentally friendly, while the enzymatic method offers various advantages such as low time, better control, and high purity. However, this method is expensive. Chemical hydrolysis is an inexpensive method to deliver the most inferior product quality (Gao et al., 2021).

FPH is obtained from fish biomass, while potential sources are cheap pelagic fish, fish by-products, and by-catches. Bitterness is often a characteristic found in FPH, which reduces their application in low sensory acceptance products. Protein hydrolysates extracted from fish material containing viscera are expected to present an even higher bitterness (Rustad, 2003). Nikoo et al. (2019) extracted FPH from farmed Rainbow trout (*Oncorhynchus mykiss*) by-products (heads, frames, viscera, and fins). They reported antioxidant activity such as radical scavenging, metal chelating, and ferric reducing antioxidant activity in fish emulsion stored for six days at 4˚C. Aspevik et al. (2016) extracted FPH from Atlantic salmon (*Salmo salar*) by-products (mix of heads and frames) using enzymatic and heat methods. FPH extracted by the heat method presented lower bitterness and higher surface-active properties than those extracted using the enzymatic process. The authors reported positive results for their potential application as emulsifiers and foaming agents.

2.1.2.3. Bioactive peptides

After the protein hydrolysis, peptides are separated by size, charge, or polarity, commonly with membrane and chromatography techniques. Subsequently, the sequence is characterized, and finally, the purity is enhanced (Gao et al., 2021). Bioactive peptides such as protamine can be used as an antibacterial agent in food preservation (Rustad, 2003). Peptides such as angiotensin-converting enzyme (ACE) inhibitory peptides found in seafood play critical regulatory roles in the human body (Suresh et al., 2018). ACE inhibitory peptides have been identified in Alaska pollock by-products, sardinella by-products, yellowfin sole frames, and tuna viscera (Gao et al., 2021; Olsen et al., 2014). Peptides from bonito have demonstrated the ability to lower blood pressure, and peptides from anchovy by-product sauce have demonstrated antioxidant activity. Bioactive peptide production and commercialization still face challenges in scale-up production, processing time and cost, peptide identification, and clinical studies for health claims (Gao et al., 2021).

2.1.2.4. Collagen

Collagen is a major structural component in connective tissues found in all animals (Dave et al., 2019; Pal & Suresh, 2016) widely used in food, pharmacy, and cosmetics. Traditionally, collagen derives from porcine and bovine sources. Some disadvantages comprise transmissible diseases, immunogenicity, religious constraints, and high cost (Nakkarike et al., 2010; Olsen et al., 2014). In this regard, marine collagen represents a low-cost and free-from-religious-constraints alternative (Zhou et al., 2020). Marine collagen can be isolated from the skin, scales, bones, cartilage, swim bladder, and fins of a wide range of finfish such as catla, rohu, seabass, carp, salmon and cod (Dave et al., 2019; Pal & Suresh, 2016), and other seafood such as cuttlefish, octopus, squid, jellyfish, and sea urchin (Suresh et al., 2018). Dave et al. (2019) quantified the

concentration of collagen in salmon fins, skin, scales, and frames; cod skin, fins, and frames; and lumpfish skin, fins, and whole-body through sulfuric acid hydrolysis, and reported a significant amount of collagen (51.11 and 27.45% of dry matter) in salmon scales and skin, respectively. The collagen concentration in salmon fins, cod skin and fins, and lumpfish skin, fins, and whole-body ranged from 10 to 20% of dry matter, while the lowest collagen concentration was found in salmon and cod frames (less than 10% pf dry matter). The authors confirmed the availability of collagen in salmon skin and scales as a potential source for high-value collagen products. Numerous benefits have been attributed to collagen and collagen hydrolysates, such as antioxidant activity, bone, and joint cell disease treatment, wound healing, antifreeze, and neuroprotective activity (Pal & Suresh, 2016). Zhou et al. (2020) extracted collagen from tilapia (*Orechromes niluticos*) skin and evaluated chondrogenic and osteogenic differentiation in vitro and cartilage and subchondral bone repair in a rabbit model. The authors reported collagen from tilapia skin as a potential biomaterial for tissue regeneration. Chandika et al. (2021) studied the properties of collagen extracted from the skin of *Paralichthys olivaceus* (an abundant and economically important species in South Korea) and performed an in-vitro and a mouse model study, and reported an efficient adhesion and proliferation in fibroblasts and keratinocytes and rapid healing and dermal tissue regeneration. The collagen extracted from *P. olivaceus* skin was suggested as a potential alternative for tissue regeneration. Zamorano-Apodaca et al. (2020) extracted collagen from by-products (mix of skin, heads, and skeleton) from a combination of fish species (sharks, mullet, guitarfish, weakfish, snapper, ray, squid, seabass, pompano dolphinfish) using Alcalase, followed by ultrafiltration. The authors obtained 5 peptides from the hydrolysate with antioxidant and antimicrobial activity, solubility, foaming, and emulsifying properties. They purported the potential application of peptides from fish by-products in the food, pharmaceutical, and medical

industries. Mineral chelating peptides have been produced from marine fish bone and fish skin collagen peptides. The chelation is induced by immersing the peptide in a solution with the mineral, followed by isolation, purification, and identification processes. The resulting peptides showed higher transport efficiency and higher absorption of minerals in in-vitro and animal model studies than inorganic sources (Luo et al., 2020). These results present the potential of mineral chelating peptides as dietary mineral supplements. Nevertheless, further studies of human mineral absorption and improvements in the production process are required (Luo et al., 2020).

2.1.2.5. Enzymes

Fish materials are essential sources of endogenous enzymes, especially digestive enzymes and lipases that can be extracted as high-value biomolecules (Dave & Routray, 2018). Fish viscera provides collagenases, trypsin, chymotrypsin, elastase, and carboxypeptidase (Nakkarike et al., 2010). The high activity at low temperatures of enzymes from cold-adapted fish has increased the interest for the application of these enzymes in different industries in processes such as fish and squid deskinning, fish roe cleaning, caviar production, fish descaling, cheese production (rennet substitute), and protein hydrolysate production (Shahidi & Ambigaipalan, 2015).

Fish by-products and especially digestive organs are sources of lipases (Kurtovic et al., 2009). Lipases have been extracted from fish digestive organs such as stomach, pyloric cecum, pancreas, and intestine from sardine, mackerel, Atlantic cod, Atlantic salmon, and Chinook salmon; and from other fish organs such as red muscle, liver, and adipose tissue from rohu, rainbow trout and juvenile rainbow trout (Kurtovic et al., 2009; Sae-Leaw & Benjakul, 2017). Although fish lipases have been studied less than lipases from microbial, plants, and animals (Kurtovic et al., 2009), fish lipases are characterized by high cold-adaptation, high stability, high catalytic activity, and salt

tolerance, with potential in several food applications. (Sae-Leaw & Benjakul, 2017). Fish lipases have been utilized in cleaning products, modified foods, flavor enhancement, and large-scale processes, including hydrolysis, transesterification, and synthesis of structured lipids (Kurtovic et al., 2010).

Kurtovic et al. (2010) purified lipases from pyloric caeca of Chinook salmon (*Oncorhynchus tshawytscha*) and Hoki (*Macruronusnovaezealandiae*) and reported the highest activity at 35˚C and pH 8-8.5. These lipases presented higher stability to acidic conditions than other fish lipases. Smichi et al. (2013) purified lipase from golden grey mullet (*M. auratus*) viscera, reporting it as a serine enzyme from a new group of fish digestive lipases. The lipase was named gray mullet digestive lipase (GmDL). It was characterized as a thermo-active enzyme active in long and short triacylglycerides and phospholipids and resistant to high salt concentrations. Sae-leaw and Bejankul (2018) purified a liver lipase from seabass (*Lates calcarifer*) with optimal activity at pH 8 and 50˚C. An experiment at 30˚C, for 3 hours at 0.3 U/g of dry seabass skin, resulted in 84.57% of skin lipids, showing higher efficiency than isopropanol, proving potential use in fat removal from fish skin.

2.1.2.6. Calcium and phosphorus

Fishbone, primarily generated during the filleting process, comprises organic and inorganic fractions containing mainly collagen and calcium phosphates (Shavandi et al., 2019). Calcium is principally present in fishbone in the form of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and calcium carbonate ($CaCO_3$) (Nawaz et al., 2020). Traditionally, fishbone powder, rich in calcium and collagen, is obtained through boiling the fish material, followed by a grinding process (Jafarpour et al., 2020). Alternatively, alkaline solubilization is included in the process, while some other

approaches are under investigation, such as enzymatic, autoclave, and particle reduction. Jafarpour et al. (2020) studied the calcium and phosphorus content in cod (*Gadus morhua*) frame powder comparing a heat process at 95˚C and an enzymatic process with Neutralase and Alcalase at 50˚C for 3 hr at 1.5% (enzyme-substrate). The authors reported significantly higher inorganic content in the powder obtained enzymatically (586 and 330 g/kg of Calcium and phosphorus), respectively. Nawaz et al. (2020) investigated fishbone powder from grass carp (*Ctenopharyngodon idella*), where bones were manually separated. Fishbone powder was obtained through heat process at 105˚C and autoclave process at 121˚C and 15 psi for 1 hr. They performed an additional procedure for particle size reduction with a high-speed dispenser followed by a colloidal mill. The authors reported a significantly higher amount of calcium and higher in vitro digestibility from the fishbone powder obtained using autoclave compared to powder obtained with heat. Interestingly, the particle size reduction increased in vitro digestibility in both methods, showing a significant role of the particle size on calcium digestibility. The authors determined the need for further studies to evaluate in vivo digestibility.

Traditionally, hydroxyapatite has been obtained from synthetic and organic sources (Terzioğlu et al., 2018). Synthetic sources often represent an expensive and time-consuming process, while organic sources are obtained by calcination and are preferred for potential higher compatibility (Terzioğlu et al., 2018). The organic hydroxyapatite extracted from livestock and wild animals bones carries potential safety and religious concerns, while fishbone is a readily available low-cost material with several advantages such as non-toxicity, non-inflammatory behavior, high biocompatibility, and no religious concerns, providing a potential use in dental implants, bone defect healing, and tissue engineering (Terzioğlu et al., 2018).

2.2. Deterioration of raw fish materials

Prolonged time under poor storage conditions such as inadequate temperature, oxygen availability, and humidity promotes the deterioration of fish materials. Fish deterioration is a sequential degradation process involving four stages: rigor mortis, resolution of rigor mortis, autolysis, and microbial spoilage (Singh & Benjakul, 2018). The first two stages of degradation are promoted by enzymatic activity, and the last two are related to microbial activity, resulting in fish spoilage (Abbas et al., 2009).

2.2.1. Enzymatic degradation

Autolysis is the self-degradation of tissues produced by endogenous enzymes (Abbas et al., 2009). It occurs during the first steps of fish deterioration. The distribution of autolytic enzymes in fish tissues and the activity upon fat and proteins derives from a complex process involving many mechanisms resulting in the degradation of fish tissues (Mukundan et al., 1986). Nevertheless, it is widely recognized that this relies on the action of endogenous proteases (Yarnpakdee et al., 2009). Following fish death, several biochemical changes associated primarily with the activity of proteases can occur. Fish proteases, found in fish muscle and digestive organs, can be classified according to the site of cleavage (endo or exopeptidases), extractability (sarcoplasmic and myofibril-associated proteases), similarity to a known protease (trypsin-like, cathepsin-like), or many other aspects such as pH sensitivity, substrate specificity, mode of catalysis and active site (Singh & Benjakul, 2018). Gastrointestinal enzymes disrupt the stomach wall and cavity during storage, releasing protein, lipids, blood, and water. Correspondingly, muscle tissue proteases such as cathepsins degrade myofibrillar proteins and collagen, resulting in tissue softening promoting

microbial growth. The deterioration is faster in fatty fish due to the high contents of lipids, myoglobin, sarcoplasmic protein, and heat-activated proteases (Singh & Benjakul, 2018).

Yarnpakdee et al. (2009) studied the autolysis of goatfish (*Mulloidichthys martinicus*) mince and analyzed the effect of a washing step and skin inclusion in the mince autolysis. The authors reported increased autolysis in the skin-containing mince and non-washed mince than washed mince. Serine and cysteine were the primary contributors to degradation. Nikoo et al. (2021) studied the autolysis of rainbow trout (*Oncorhynchus mykiss*) by-products under different hydrolysis conditions and obtained hydrolysates rich in small peptides at 40˚C for 1 hr. They also reported increased peroxides and TBARS in lipids and hydrolysates associated with PUFA oxidation.

2.2.2. Microbial spoilage

Microbial activity is the primary cause of raw fish spoilage. Typical bacteria found in raw fish include *Pseudomonas spp, Alcaligenes, Vibrio, Serratia, Micrococcus, Pseudomonas spp, and Shewanella spp*, generating compounds as amines, organic acids, sulfides, alcohols, aldehydes, ketone, and trimethylamine (TMA) (Ghaly et al., 2010). Good manufacturing practices (GMP) determine 10^6 CFU/g as the total viable count (TVC) limit. However, the sensory limit for rejecting raw fish can be found below that count, suggesting a lack of correlation between total viable limit and sensorial end of shelf life in fish (Mikš-Krajnik et al., 2016). For this reason, specific spoilage organisms (SSO), which are the organisms contributing to spoilage, and volatile organic compounds (VOC) have been used to better estimate the threshold for microbial spoilage of fish (Mikš-Krajnik et al., 2016). Milks-Krajnik et al. (2016) studied the shelf life of raw Atlantic salmon fillets stored for 14 days at 4 and 10˚C and 72 hr at 21˚C and reported *Pseudomonas spp*

as the SSO suitable for shelf-life determination and correlated with the formation of 3Met-1 But and aldehydes. Churchill et al. (2016) studied the shelf life of head-on and gutted Atlantic salmon stored at 0, 5, 10, and 15˚C and reported a positive correlation between TVC and sensory quality index in samples stored at 5, 10, and 15˚C, with a low correlation at 0˚C. This study predicted the time to reach a TVC of 10^7 cfu/g at 0, 5, 10, and 15˚C, being 11.1, 7.2, 4.4, and 2.4 days, respectively. Fogarty et al. (2019) evaluated farmed Atlantic salmon stored aerobically at 2˚C and reported the end of shelf life at 5-6 $\log_{10}$ cfu/g in 10 days. The authors reported hydrogen sulfide producing bacteria (HSPB), lactic acid bacteria (LAB), *Pseudomonas spp, Brochothrix thermosphacta, and Photobacterium spp* as better indicators of fish spoilage compared to TVC.

2.2.3. Lipid deterioration

Lipid oxidation and lipolysis are two biochemical reactions strongly associated with organoleptic changes and deterioration of foodstuffs (Xu et al., 2019). Oxygen and the double bonds of lipids are typically involved in oxidation (Ghaly et al., 2010). Lipases and phospholipases are the main enzymes in lipolysis, which cause the release of free fatty acids (Xu et al., 2019). The development of free fatty acids in foodstuffs from lipid oxidation and lipolysis typically co-occurs (Xu et al., 2019). These reactions enhance flavor in foods such as fermented meats at the low range, while excessive lipolysis and lipid oxidation result in product deterioration (Xu et al., 2019).

2.2.3.1. Lipid oxidation

Lipid oxidation is a significant cause of food deterioration, especially food high in PUFA, such as fish (Tatiyaborworntham et al., 2021). The process of lipid oxidation includes two stages: peroxides are formed during the primary stage, while lower-weight compounds such as aldehydes and ketones are formed in the second stage (Medina & Pazos, 2010). During the primary oxidation

stage, the reactions are autoxidation, photo-oxidation, and enzymatic oxidation, leading to the formation of peroxides (Hultin, 1994; Medina & Pazos, 2010). Autoxidation is the principal process in lipid oxidation and is developed in three steps: initiation, propagation, and termination (Medina & Pazos, 2010). During initiation, the molecular oxygen, which is present in all organisms, undergoes several reductions generating reactive oxygen species (ROS), which subsequently interact with fatty acids present in the cell as triacylglycerol and cell membrane as phospholipids, resulting in the formation of lipid radicals (Medina & Pazos, 2010). The radical hydroxyl has been hypothesized to be the primary ROS initiator of lipid oxidation (Hultin, 1994). Oxygen is attached to the lipid radicals during propagation, promoting a chain reaction that generates different free radicals and hydroperoxides (O'Keefe & Pike, 2010). Subsequently, the free radical interaction forms non-radical structures (Ghaly et al., 2010). During photo-oxidation, a molecule of oxygen is attached to a fatty acid double bond in a process that requires light and a photosensitizer. Photosensitizers are molecules such as chlorophyll and hemoproteins capable of absorbing light that reacts with molecular oxygen, catalyzing the formation of singlet oxygen, a highly unstable form of oxygen that reacts with lipids (Dogra & Kim, 2020). During enzymatic oxidation, lipoxygenases directly catalyze the addition of an oxygen molecule to a fatty acid double bond (Medina & Pazos, 2010). The fragmentation of lipid hydroperoxides to lower molecular weight products such as aldehydes and ketones, alcohols, and hydrocarbons occurs during the second oxidation stage (O'Keefe & Pike, 2010; Tejero et al., 2004).

The lipid oxidation state in food materials can be determined using a variety of procedures. Table 1 summarizes the principal analysis performed to assess lipid oxidation (O'Keefe & Pike, 2010) and the permissible limits designed by the Codex Alimentarius (Codex Alimentarius Commission, 2017).

Table 1. Lipid oxidation analysis and quality limits by the Codex Alimentarius (2017*)* (O'Keefe & Pike, 2010).

Oxidation stage	Oxidation analysis	Analytic principle	Permissible limit
The primary stage of oxidation	Peroxide value	Peroxides react with potassium iodide with the formation of iodine which is then quantified by titration	5 meq peroxide/kg oil
	Conjugated diene and triene	Conjugated dienes absorb UV light at 232 nm and trienes at 270 nm and are quantified with a spectrophotometer	NA
The secondary stage of oxidation	*p*-Anisidine value	Aldehydes react with *p*-Anisidine reagent with the formation of chromogen that is quantified spectrophotometrically at 350 nm	20
	TBARS (Thiobarbituric acid reactive substances)	Malonaldehyde reacts with Thiobarbituric acid generating a color that is measured in a spectrophotometer at 530 nm	NA
	Volatile organic compounds	Volatile compounds such as hexanal are calculated from the peak area of a chromatogram of static headspace analysis of the sample and a standard	NA
Total oxidation	TOTOX	Total oxidation is analyzed from the peroxide and *p*-Anisidine values (TOTOX=2PV+AV)	26

NA: No limit available.

Hydroperoxides are formed during the first stage of lipid oxidation, resulting in non-conjugated double bonds transformed into conjugated dienes and trienes, which absorb UV light at determined absorbance.In an advanced oxidation state, secondary oxidation products such as malonaldehyde and hexanal are formed. Aldehydes can be analyzed with *p*-Anisidine, malonaldehyde-type compounds with TBARS, and organic compounds such as pentane, pentanal, and hexanal are analyzed with Gas Chromatography equipped with flame ionization detector (GC-FID) or mass selective detector (GC-MS). Given the dynamic nature of the oxidation process, at least two different analyses must determine the extent of lipid oxidation in a sample. The TOTOX value is a measure of the total oxidation in a sample. This value considers the primary and secondary stages of lipid oxidation, resulting in a more accurate estimation of lipid oxidation. The TOTOX value is constructed by analyzing Peroxide value and *p*-Anisidine value in the equation 2PV+AV (O'Keefe & Pike, 2010).

2.2.3.2. Lipolysis

Lipolysis is the hydrolysis of lipids induced by the endogenous enzymes *lipases* with the generation of free fatty acids (different from enzymatic oxidation which generates peroxides) (Xu et al., 2019). Lipases catalyze the hydrolysis of ester bonds in structures such as triacylglycerides, phospholipids, cholesteryl-esters, and vitamin-esters and are found in fish viscera, skin, and digestive organs (Kurtovic et al., 2009).

Lipases play an essential role in the degradation of fish after slaughter and during processing and storage in chilling and freezing conditions (Smichi et al., 2013). The release of free fatty acids promotes faster oxidation in foodstuffs (Sae-Leaw & Benjakul, 2017). It is suggested that the pro-oxidant activity of free fatty acids is related to their ability to migrate to the lipid-water interfaces

in the matrix, decreasing the surface tension of the oil droplet and increasing the contact between oxygen from air and oil (Liu et al., 2021; Waraho et al., 2011).

2.3. Preservation of fishery products

Enzymes, bacteria, and oxygen are the leading causes of fish deterioration. The degradative process starts from the point of fish harvest and subsequently is influenced by the handling and preservation practices applied (Abraha et al., 2018). Different preservation methods such as drying, smoking, salting, freezing, and canning are used for fishery products. However, high temperatures in many of these preservation methods promote protein thermal denaturation, aggregation, and destruction of cell membranes, resulting in a significant undesirable texture and physiological changes in products (Abraha et al., 2018). A combination of techniques should be applied to preserve the natural chemical and physiological characteristics of fish and fish by-products, such as low temperature, modified atmospheres, and additive addition to ensure quality and microbial safety. This approach is known as hurdle technology (Yesudhason et al., 2014). Low-temperature storage and oxidation inhibitors are among the most critical strategies for controlling autolytic, oxidative, and microbial deterioration in fish and fish products (Ghaly et al., 2010). Table 2 lists the principal preservation methods applied to fish and fish products. The following subsections illustrate the main preservation methods commonly employed for fish by-products.

2.3.1. Low-temperature storage

According to the optimal growth temperature, microorganisms are classified in psychrophiles (<15˚C), mesophiles (30-37˚C), and thermophiles (>50˚C). However, these microorganisms can survive at -20 to 20˚C, 20 to 45˚C, and 20 to70˚C, respectively (Cheng et al., 2021).

Table 2. General methods for fish products preservation.

Method	Description	Parameters	Merits	Demerits	Reference
Chilling	Storage at low temperatures with the use of ice flakes, ice slurries, or chilled seawater	0-4˚C	A convenient method for fish preservation	Microbial, enzymatic, and oxidative spoilage occurs	(Hussain et al., 2021)
Freezing	Storage at freezing temperatures for extended periods	-18 to -30˚C	The primary method for fish preservation inhibiting microbial spoilage	Enzymatic and non-enzymatic reactions occur. Ice crystals can damage the texture and enhance oxidation	(Hussain et al., 2021)
Canning	A heating process under pressure in tight containers	116-130˚C (Commonly 121˚C)	A traditional method providing 1 to 5 years of shelf life	Affects texture, flavor, and nutritional value. Risk of oxidation in oily fish	(Sampels, 2015)
Smoking	Smoke production by combustion or electrostatic smoking, including heating, drying, and salting	Cold smoking <33˚C, hot smoking 80-100˚C	Traditional method with phenolic compounds generation providing antimicrobial and antioxidative effects	Lipid oxidation can occur. The process affects texture. Used more as a new product process than as a preservative process	(Sampels, 2015)
Drying	Partial removal of water and reduction of water activity	Sun drying, air drying, oven drying, solar tunnel	Decreases bacteria and enzymatic activity, delaying quality deterioration	Lipid oxidation occurs	(Sampels, 2015) (Giannakourou et al., 2020)
Salting	Dehydration of product through osmosis by the addition of salt	Water activity of 0.8-0.7	Decrease of enzymatic and bacterial activity by reduced water activity and salty environment	Protein degradation and oxidation still occur	(Hussain et al., 2021) (Sampels, 2015

Method	Description	Parameters	Merits	Demerits	Reference
Irradiation	Exposition of product to ionizing radiation from electron beams, x-rays, or gamma rays	0.1-10 kGy	Removal of pathogens and microbial spoilage by DNA damages and cell lysis	Nutritional value, quality, and sensory loss, along with consumer acceptance concerns, exist	(Aaliya et al., 2021)
High-Pressure Processing	High-pressure processing where a product is submitted to high pressure at ambient temperature	100-1000 Mpa	Inactivation of microbes by DNA syntheses inhibition, enzymes inactivation, protein denaturation. Alternative to thermal processing with an extended shelf life of products	Texture change, lipid oxidation, and protein denaturation can occur. Spores can survive	(Aaliya et al., 2021) (Hussain et al., 2021)
Modified Atmosphere Packaging	A combination of gases inside the package to inhibit the growth of undesired microbial	Commonly oxygen, carbon dioxide, and nitrogen	Microbial and chemical reactions are inhibited, applied to fresh food resulting in extended shelf life	Demand for environmentally friendly alternatives	(Hussain et al., 2021)
Chemicals	Addition of antimicrobial and antioxidant compounds	Sorbates, benzoates, propionates, nitrates, sulfites, parabens, BHA, BHT, TBHQ	Delay in the microbial and chemical reactions resulting in extended shelf-life	Toxicity related to the use of chemicals and synthetic preservatives	(Hussain et al., 2021)
Natural preservatives	Addition of antimicrobials and antioxidants from microbial, plant, or animal sources	Bactericides, plant extracts, chitosan, enzymes, peptides	Natural preservatives are non-toxic and can be effective alternatives to synthetic compounds	Natural preservatives can impart mild taste and color and be cost-effective	(Hussain et al., 2021)

A cold environment promotes reduced activity and microbial destruction, and low-temperature storage is a primary method to inhibit and control microbial growth (Cheng et al., 2021). Low-temperature storage includes refrigeration, super chilling, and freezing. Refrigerated storage comprises the temperatures above the freezing point of water (0˚C). In comparison, super chilling storage situates near the initial freezing point (-1 to -2˚C), and frozen storage temperature starts at -18˚C and below (Wang et al., 2018). The commercial freezing temperature for food products is considered -18˚C (Ghaly et al., 2010; Vázquez et al., 2013; Wang et al., 2018). Although frozen storage preserves foodstuffs for a longer time compared to refrigerated and superchilled storage, product quality deterioration of frozen seafood proceeds due to cell and muscle destruction caused by ice crystals, enzyme activity, freezing rate, and oxidation still occurring under freezing conditions, although at a slower rate (Abraha et al., 2018; Ghaly et al., 2010; Yu et al., 2021). Karlsdottir et al. (2014) studied the lipid oxidation development in light and dark muscle of saithe (*Pollachius virens*) and Hoki (*Macruronus novaezelandiae*) stored for 18 months at -20 and -30˚C. In this study, the lipid content of light muscle in both species was 0.6%, while dark muscle of saithe and Hoki was 1.1% and 7.6%, respectively. The dark muscle developed higher lipid oxidation compared to light muscle in both species. All treatments developed higher lipid oxidation at -20˚C compared to -30˚C. The Hoki dark muscle stored at -20˚C developed the most elevated lipid oxidation across species, in addition to being 6 times higher compared to its light muscle stored at the same temperature. Storage at -30˚C was recommended for a longer storage life of these species. Tolstorebrov et al. (2014) investigated the lipid oxidation development in Atlantic herring (*Clupea harengus*) fillets during long-term storage at -25 and -45˚C and reported a significant oxidation inhibition at -45˚C. Yu et al. (2021) studied the effect of ice crystals, endogenous proteolysis, and lipid oxidation on the quality degradation of pufferfish (*Takifugu*

obscurus) fillets stored at -18˚C for 18 months and reported the destruction of cells and muscle due to the formation of higher diameter of ice crystals during slow freezing as opposed to the diameter of ice crystals obtained with liquid nitrogen freezing. They concluded that the diameter of ice crystals affects the degradation rate of fish fillets. The small crystals formed with liquid nitrogen resulted in lower development of trimethylamine and lower flavor loss in fillets during storage. Torres-Arreola et al. (2007) stored fillets of sierra fish (*Scomberomorus sierra*) at -25˚C for 120 days. They reported a significant lipid oxidation development (PV 15.15 meq/kg, TBARS 11.95 mg malonaldehyde equivalents/kg, FFA 11.83%) in samples packaged in low-density polyethylene (LDPE), which subsequently resulted in texture damage according to muscle cell detachment degree, fractures in muscle fibers, and evident fiber deformation after 120 days of storage. The loss of firmness reported in the share-force analysis was linked to protein denaturation. Vazquez et al. (2013) investigated lipid hydrolysis and lipid oxidation in frozen Atlantic mackerel (*Scomber scombrus*) stored at -10˚C for three months and reported lipid hydrolysis (FFA 4.1%) and mild oxidation in primary, secondary, and tertiary oxidation stages (PV 2.97 meq/kg, TBARS 0.88 mg malonaldehyde equivalents/kg, fluorescence formation 0.82, respectively). The authors reported high hydrostatic pressure (HHP) as a promising technology to partially inhibit lipid degradation at 150-450 MPa of pressure and 0-5 min of holding time. Free fatty acids and tertiary oxidation products were reduced as high pressure and time increased.

Duun and Rustad (2008) studied the shelf life of superchilled and ice-stored farmed Atlantic salmon fillets. The superchilled fillets were stored in cold rooms at -1.4 and -3.6˚C after vacuum packaging while, the ice-stored fillets were directly stored on ice (approximately 0˚C) after vacuum packaging. In this study, the shelf life of the superchilled fillets increased to twice that of ice-stored fillets. Shumilina et al. (2016) evaluated Atlantic salmon backbones, heads, and viscera

as sources of bioactive metabolites (anserine, phosphocreatine, and taurine) and analyzed their quality during 7 days at 4 and 10˚C, respectively. The maximum storage time of 7 days was reported at 4˚C, while 3 days at 10˚C according to biogenic amines and harmful compounds formation and bioactive metabolite degradation. Dave and Routray (2018) studied the quality of freeze-dried salmon by-products (a mix of heads, frames, and viscera) stored at 5˚C and -30˚C for 35 days, and reported a low water activity, low microbial growth, and slight changes in color in all samples during the storage period at both temperatures, concluding favorable conditions for the storage of salmon by-products for a short period. During the same study, the fatty acid profile and oil yield of salmon frames harvested at different times (over a timespan of 9 months) and stored at -30˚C was assessed. In this part of the study, the oil yield and the monounsaturated fatty acids decreased, the saturated fatty acids increased, and the PUFAs remained less affected during storage. The authors concluded that -30˚C was a favorable condition for preserving the quality of the oil extracted from the fish frames. Wu and Bechtel (2008) analyzed the quality of Alaska pink salmon (*Oncorhynchus gorbuscha*) by-products (heads and viscera combined) stored for 4 days at 6 and 15˚C in terms of lipid hydrolysis, lipid oxidation, and endogenous antioxidant activity. A significant generation of FFA (7%) was reported with increased time and storage temperature during the study. The antioxidant activity was reduced to approximately 25% of the initial level with time. The authors suggested the viability of the storage of salmon by-products at 15˚C for 4 days. Wu and Bechtel (2009) analyzed the quality of Walleye pollock (*Theragra chalcogramma*) by-products (head, skin, frames, and viscera combined) stored for 4 days at 15˚C and 10 days at 6˚C. And reported higher lipid oxidation at 15˚C. FFA increased to 7% after the 4^{th} and 8^{th} day at 15˚C and 6˚C, respectively. The percentage of PUFA remained constant during the study at both storage conditions, demonstrating an absence of degradation of these fatty acids. However, the

content of endogenous tocopherol in by-products decreased at a similar rate during both storage temperatures. Aidos et al. (2003) analyzed Herring (*Clupea harengus*) by-products (heads, frames, skin, and viscera combined) stored at 2 and 15˚C for 72 hr and reported a higher deterioration indicated by total volatile bases (TVB), biogenic amines formation, and FFA increase in by-products stored at 15˚C. However, the percentage of PUFA in the samples remained constant during the study.

2.3.2. Limiting oxygen

Molecular oxygen is the substrate initiator of the lipid oxidation process (Hultin, 1994). For this reason, reducing the sources of oxygen and reducing the oxygen available to react with lipids is paramount for the oxidation rate decrease (Hultin, 1994; Medina & Pazos, 2010). The strategies to limit molecular oxygen contact with the product during storage rely on the packaging and the oxygen available. The principal techniques for limiting oxygen include vacuum, nitrogen, modified atmospheres (MAP), and high-barrier packaging materials. This solution has increased the shelf life of fish products compared to air-packaging or no-packaging due to the decrease of oxygen available to oxidize lipids. Tolstorebrov et al. (2014) evaluated lipid oxidation in Atlantic herring (*Clupea harengus*) fillets packaged with high-barrier and low-barrier packaging materials and stored at -25 and -45˚ for one year. Lipid oxidation increased in the samples stored at -25˚C and packaged with a low-barrier material. Lipid oxidation was inhibited in samples stored at -45˚C regardless of the packaging material and stored at -25˚C packaged with the high-barrier material. The authors concluded that oxygen availability was the principal factor in lipid oxidation development. Sone et al. (2012) analyzed the lipid oxidation development and lipid degradation of Atlantic salmon fillets stored at 4˚C for 16 days under three different packaging conditions: low barrier, modified atmosphere (60% CO_2, and 40% N_2), and vacuum. A higher lipid degradation

was reported in samples stored under the low-barrier packaging material, while the lowest lipid degradation was reported with MAP. Remya et al. (2017) studied the effect of packaging methods consisting of control (no antimicrobial film or oxygen scavenger film), antimicrobial film, oxygen scavenger film, and combined antimicrobial-oxygen scavenger film materials on the shelf life of farmed cobia (*Rachycentron canadum*) steaks stored at 2˚C for 30 days. The shelf-life provided by the different packaging methods was higher in the steaks packaged with combined (antimicrobial-oxygen scavenger) materials, followed by oxygen scavenger, antimicrobial, and finally by the steaks packaged with the control (no antimicrobial nor oxygen scavenger) materials. The shelf life of steaks was increased by 15 days with the combined (antimicrobial-oxygen scavenger) packaging method compared to control (no antimicrobial or oxygen scavenger). The available oxygen was found to be the principal factor for the lipid degradation of cobia steaks. Hassoun and Karoui (2015) evaluated the combined effect of light (7 watts of light and dark conditions) and oxygen (partial and total vacuum packaging) on the degradation of whiting fish (*Merlangius merlangus*) fillets stored at 4˚C for 12 days. The exposure of fillets to 7 W light during storage was reported to be the principal factor promoting lipid oxidation. Lipid oxidation increased with the combined effect of exposure to light (7 watts) and exposure to oxygen (partial vacuum) storage conditions, while it was restrained with the combined effect of absence of light (dark) and absence of oxygen (total vacuum) storage conditions.

2.3.3. Antioxidants

Antioxidants are compounds that either prevent oxidation catalysts or are preferentially oxidized before lipids (Torres-Arreola et al., 2007). These compounds control the rate of lipid oxidation found in PUFAs (Huber et al., 2009). The most popular antioxidants used in food products comprise butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butyl

hydroquinone (TBHQ), which are synthetic phenol derivatives with high free radical scavenging activity (Ghaly et al., 2010; Huber et al., 2009; Li et al., 2020). Synthetic antioxidants effectively constrain lipid oxidation; nevertheless, natural antioxidants are preferred for the potential toxicity and carcinogenesis related to the use of synthetic antioxidants (Huber et al., 2009; Li et al., 2018; Zhang et al., 2010). Phenolic compounds have been extracted from natural sources such as fruits, vegetables, and spices. These compounds include flavonoids, polyphenols, and terpenoids. Carnosic acid is a lipophilic diterpene present in rosemary leaves, scavenging oxygen, hydroxyl radicals, and lipid peroxyl radicals (Basappa Maheswarappa et al., 2014; Wang et al., 2011; Zhang et al., 2010). Navarro-Garcia et al. (2017) studied the effect of tocopherol (0.5%), caffeic acid (0.06%), rosemary extract of 2.5%, and TBHQ (0.02%) on lipid oxidation of Raya (*Rhinoptera bonasus*) liver oil in an accelerated study (storage at elevated temperature conditions) at 60˚C for 35 days. Initial hydroperoxides in the Raya liver oil were 2 meq/kg, increasing at a low rate under the limit of 5 meq/kg for the first 14 days of storage in all the treatments, after which the lipid oxidation rate increased over the quality limit, except in the sample with rosemary extract added. Lipid oxidation in the samples with rosemary extract added performed a lag phase during the first 21 days, after which lipid oxidation increased to more than 5 meq/kg. The samples with rosemary extract added exhibited 7 more days of shelf-life than every other treatment. In this study, 7 days of storage at 60˚C correspond to 7 months of storage at room temperature. Zhang et al. (2010) evaluated the effect of carnosic acid (25, 60, and 98% of concentration), BHA, BHT, and TBHQ (200mg/g) on the lipid oxidation of sunflower oil stored at 60˚C for 21 days. All treatments significantly reduced lipid oxidation compared to control (no antioxidant added). The increased concentration of carnosic acid in the oil resulted in a lower lipid oxidation development. The samples added with carnosic acid developed lower lipid oxidation than BHA and BHT. The lowest

lipid oxidation was reported in TBHQ samples and was comparable to that obtained with carnosic acid at a concentration of 98%. Wang et al. (2011) studied the effect of carnosic acid (0.1, 0.2 and 0.3 mg/g), vitamin E (0.2 mg/kg), and TBHQ (0.2 mg/kg) on the lipid oxidation of fish oil stored at 30 and 4˚C for 66 days and reported reduced lipid oxidation with the increase of carnosic acid content. Carnosic acid samples developed lower lipid oxidation compared to vitamin E samples. The lowest lipid oxidation development was reported in the TBHQ samples. The addition of TBHQ at 0.2 mg/kg and carnosic acid at 0.3 mg/g promoted the stability of fish oil to lipid oxidation. However, the PUFA content was higher in the samples with carnosic acid added at 0.3 mg/g. Li et al. (2018) evaluated the effect of carnosic acid at different concentrations (20, 60, and 99%) and BHA, BHT, and TBHQ at a 0.02 mg/kg concentration on the lipid oxidation of tara oil stored at 60˚C for 15 days. The lowest lipid oxidation development was reported in samples with TBHQ added and 99% carnosic acid, followed by 60% carnosic acid, 20% carnosic acid, BHA, and BHT. The authors reported carnosic acid as an alternative to synthetic antioxidants with similar efficiency to TBHQ.

Feeding diets high in natural antioxidants such as tocopherols and astaxanthin to farmed fish provides higher resistance of fish material to lipid oxidation during storage. Jones and Carton (2015) analyzed the effect of a high-tocopherol diet (628 mg α-tocopherol acetate/kg) compared to control (192 mg α-tocopherol acetate/kg) on the lipid oxidation of farmed barramundi (*Lates calcarifer*) whole fish and fillets stored 14 days at 2˚C. Lipid oxidation was lower in the high tocopherol fish, although no significant difference was reported as the range of oxidation developed was low. Jensen et al. (1998) studied the effect of different levels of dietary fat (27 and 32%), dietary α-tocopherol (100 – 600 mg/kg), and dietary astaxanthin (40-100 mg/kg) on the lipid oxidation of gutted farmed rainbow trout (*Oncorhynchus mykiss*) stored at -28˚C for 18 months

and smoked fillets stored at 3˚C for 3 weeks prepared from the 18-month-stored gutted fish. The authors reported a lower lipid oxidation rate in samples fed diets high in antioxidants than the samples fed low antioxidants diets. During the study of frozen fish stored at -28˚C, high levels of astaxanthin from the diet resulted in lower lipid oxidation. While during the study of smoked fillets stored at 3˚C, the high levels of α-tocopherol from the diet resulted in lower lipid oxidation of fillets during storage. The authors suggested a more substantial influence of astaxanthin activity in the early stages of lipid oxidation, while tocopherol influenced the later oxidation stages.

2.4. Processing of fish by-products

The type of processing applied to fish materials affects the rate of oxidative deterioration occurring during handling, storage, and further processing, due to increased exposure of membrane and storage lipids to pro-oxidant compounds such as oxygen, enzymes, and metals contained in the fish (Hultin, 1994; Medina & Pazos, 2010). The lipid oxidation rate in fish products is influenced by the degree of tissue disruption (Hultin, 1994). Mild processing such as filleting, and processes where the fish materials are kept intact (unprocessed fish), allows a higher degree of tissue integrity (Medina & Pazos, 2010). In contrast, high processing such as mincing and grinding, in which the fish tissues are reduced to a homogenized mass, results in higher tissue disruption (Hultin, 1994).

2.4.1. Screening of highly degradable tissues

Fish parts, organs, and tissues may present different chemistry, composition, enzymatic systems, and antioxidant/pro-oxidant systems; therefore, they should be studied separately to understand lipid oxidation (Rustad, 2003; Wu et al., 2022). The lipid oxidation in fish skin, dark muscle, and white muscle are developed at different rates. Fish skin oxidizes faster than dark muscle, and dark

muscle deteriorates faster than white muscle (Hultin, 1994). For their practical preservation, fish by-products should be studied and characterized individually (Rustad, 2003).

2.4.2. Grinding and mincing

In high processing methods such as grinding and mincing, the fish tissues are broken down several times until a uniform mass is formed. During this process, the reduced tissues are exposed to more oxygen than in low/mild processes, such as filleting and unprocessed fish (Hultin, 1994). Therefore, in fish mince, the lipid oxidation rate is higher than in fish fillets and whole fish (Medina & Pazos, 2010). Shumilina et al. (2016) evaluated the degradation of whole and minced heads from Atlantic salmon stored at 4 and 10˚C for 7 days and reported a higher degradation in the minced head than the whole head at both storage temperatures. The whole head stored at 4˚C remained under the acceptable threshold of 29.5mg/100g of TMA, while the whole head held at 10˚C exceeded it by day 7. Minced head stored at 4˚C exceeded the limit by day 7 and day 3 during storage at 10˚C. The mincing of the head resulted in the reduction of storage time by 2 to 3 days. In whole heads, putrescine was reported only on day 7 during storage at 10˚C, under the permissible limit of 17mg/100g. Minced heads developed above-permissible-limit levels of putrescine by day 7 at both storage temperatures. To the best of our knowledge, no study evaluated processing methods on the lipid oxidation of fish by-products. However, the analysis of fillets and unprocessed fish provided a comparison of the effect of levels of processing on the degradation of fish, indicating a higher deterioration in fillets compared to whole fish during storage, related to a higher surface area exposition to oxygen, thus, resulting in lower shelf life. Islami et al. (2015) studied the quality of whole and filleted farmed striped catfish (*Pangasianodon hypophthalmus*) during ice storage and reported a reduction of 3 days of shelf life promoted by the filleting process as indicated by total volatile bases and organoleptic analysis. The results were attributed to the

increased exposure of fillet tissues to oxygen. Rong et al. (2009) studied the quality of whole and filleted farmed tilapia (*Oreochromis niloticus*) during storage at 5˚C, reporting significantly lower ($p<0.5$) values of TBA, lower degradation index, and acceptability in whole than filleted tilapia, resulting in the reduction of the fillets shelf life to half that of whole fish. The higher oxidation in fillets was attributed to a higher surface area exposed to oxygen and other degradative compounds. Similar findings have been reported in farmed rainbow trout and seabass, where mild processing such as filleting increases the exposure of fish lipids to oxygen, leading to reduced shelf-life (Chytiri et al., 2004; Taliadourou et al., 2003). Milk protein concentrate was reported to decrease the lipid oxidation of herring (*Clupea harengus*) during storage at -18˚C (Joaquin, 2008), while white grape antioxidant dietary fiber decreased the lipid oxidation of horse mackerel (*Trachurus trachurus*) during frozen storage at -20˚C (Sánchez-Alonso et al., 2008).

2.4.3. Addition of antioxidants

The commonly used antioxidants such as synthetic BHA, BHT, and TBHQ or the emerging natural antioxidants such as polyphenols and flavonoids (Huber et al., 2009; Zhang et al., 2010) can be added to food products through different methods. Direct methods include grinding, dipping/immersion, and tumbling (Albertos et al., 2015; Siró et al., 2009; Wu et al., 2020). Antioxidants can also be added indirectly through the packaging, and this technique is known as active packaging or intelligent packaging (Barbosa-Pereira et al., 2013; Torres-Arreola et al., 2007).

2.4.3.1. Grinding and mincing

Grinding and mincing methods allow good mixing of the antioxidant with the fish material, ensuring a homogenized distribution of antioxidants and additives into the product. Wu et al.

(2020) investigated the effectiveness of rinsing, incubation, and mincing as antioxidant-addition methods and evaluated the lipid oxidation of herring (*Clupea harengus*) by-products stored in ice pellets (and at 4˚C in the case of incubation). Two antioxidant systems were evaluated: (1) a commercial mix containing rosemary extract, ascorbic acid, tocopherols, and citric acid, and (2) a blend of isoascorbic acid and ethylenediaminetetraacetic acid (EDTA). According to the inhibition of lipid oxidation found in the treatments compared to controls, the authors reported rinsing and incubation as effective methods for adding antioxidants, which developed oxidation from day 1. The commercial antioxidant containing rosemary showed the highest antioxidant activity in the three addition methods evaluated, used at dosages of 5%, 1%, and 0.5% during rinsing, incubation, and mincing, respectively. The mincing method showed the highest effectiveness for adding antioxidants, delaying oxidation for 12 days, and lipid oxidation inhibition for 8 and 7 days with rinsing and incubation. The authors concluded that the stabilization of herring by-products during storage near 0˚ was successful in this study. Albertos et al. (2015) obtained carob seed peel extract and evaluated its antioxidant activity on stored Atlantic horse mackerel (*Trachurus trachurus*) mince. The extract was dispersed in cold water and added directly to fish mince after the grinding process, and the samples were stored in trays at 4˚C for 3 days. The analysis of peroxide value and TBARS indicated the effectiveness of the antioxidant and the addition method to reduce lipid oxidation of the fish mince. The control samples (no carob peel seed extract) showed above 5 meq/kg of peroxides and a significant increase in TBARS since day 1 of storage. Contrarily, the samples with the extract added remained stable and below 5 meq of peroxide/kg and with a significantly lower rate of TBARS than the control during storage. Saveena Farvin et al. (2012) evaluated an ethanol extract of potato peel as a natural antioxidant in minced horse mackerel (*Trachurus trachurus*) and evaluated the lipid quality during refrigerated storage. The addition of

the antioxidant was performed during grinding. After grinding the fish, the extracts were dissolved in distilled water and added directly to the mince, stored at 5˚C for 96 hr. Based on the peroxide value and volatile secondary oxidation products, the antioxidant and the addition method effectively inhibited the lipid oxidation of minced horse mackerel. While the control (no antioxidant) increased above 5 meq of peroxide/kg and showed significant development of volatile secondary oxidation products, the samples with the added antioxidant showed inhibited lipid oxidation during storage.

2.4.3.2. Dipping/ immersion

Dipping and immersion are suitable methods for adding an antioxidant to minimally processed fish such as fish fillets and whole fish. During dipping/immersion, the fish pieces are placed in a solution containing the antioxidants for a period of time from 5 to 30 min, after which, fish parts are commonly drained and packaged (Ibrahim Sallam, 2007; Miranda et al., 2018; Mohan et al., 2010; Wu et al., 2020; Yesudhason et al., 2014). Yesudhason et al. (2014) evaluated the effect of sodium acetate on the quality of seer fish (*Scomberomorus commerson*) steaks stored at 0-2˚C for 32 days. Steaks weighing 100-110 g were immersed in a 1% sodium acetate solution for 30 min, drained, packaged, and stored. The authors reported an increase of 8 days in the shelf life of samples treated with sodium acetate compared to control, demonstrating the effectiveness of immersion as an additive-addition method. During a similar study, Mohan et al. (2010) reported 5 days of extension in the shelf life of seer fish steaks (2 cm thickness and weighing 100 g) immersed for 10 min in a 2% sodium acetate solution before packaging and storing at 0-2˚C, compared to a control not immersed in the sodium acetate solution. Ibrahim Sallam (2007) studied the effect on shelf-life of sodium acetate, lactate, and citrate added through immersion to Pacific salmon (*Onchorhynchus nerka*) skin-on slices stored at 1˚C for 15 days. During this study, salmon slices

weighing 110 g were immersed for 10 min in solutions of 2.5% sodium acetate, lactate, and citrate, then drained, packaged, and stored. In this study, an extension of 4-7 days in the shelf-life of samples immersed in these salts was reported, compared to a control (immersion in distilled water). Miranda et al. (2018) evaluated the effect of algae, *Focus spiralis,* added through immersion on the degradation of megrim fish (*Lepidorhombus whiffiagonis*) stored on ice for 13 days. The whole fish was immersed in solutions 0.01 and 0.05% of alga extract for 5 min, drained, and stored on ice. The authors reported an antimicrobial effect of the algal extract in the fish to reduce aerobes, psychrotrophic, and Enterobacteriaceae activities. They also observed a reduction in FFA generation and improved sensory attributes on samples immersed in algal extract compared to distilled water and non-immersed samples.

2.4.3.3. Tumbling

The tumbling process comprises the mechanical forces to which meat tissue is subjected during a controlled rotation in a hermetically closed drum with or without the use of vacuum (Daudin et al., 2015). This process is widely used in the meat industry to promote the migration of salt and additives into the meat matrix as preservation agents and enhance meat tenderness and juiciness through protein solubilization (Siró et al., 2009). Tumbling times range from short periods to 12 hours according to the degree of structural modification and diffusivity required for the product. The use of vacuum promotes the release of occluded gases in the tissue (Li et al., 2020; Mirade et al., 2020). Tumbling is a technology developed for the meat industry, with most studies being performed on pork and beef. Siro et al. (2009) analyzed the diffusion coefficient of sodium chloride into pork loins (*Longissimus dorsi*) under vacuum tumbling, static immersion, and ultrasonic methods for 30 to 180 min. In this study, the highest diffusion was reported in the tumbling method. The highest diffusion rates were reported during the first 30 min of the process in the three

methods. Static immersion provided the lowest diffusion of sodium chloride into the meat. Mirade et al. (2020) conducted a study with beef meat (*Biceps femoris*) processed using the tumbling method, analyzing the type of tumbling (strong tumbling, provided by a 180 L industrial tumbler, and soft tumbling, provided by a 10 cm-diameter laboratory tumbler), tumbling time (15 and 60 min), and vacuum pressure (atmospheric pressure and partial vacuum) on the diffusion rate of salt and acetic acid into the meat. The authors reported tumbling type as the principal factor affecting solute diffusion, followed by tumbling time and vacuum pressure. The significant diffusion of solutes was reached during the first 15 min, where the salt and acetic acid penetrated the first 2-3 mm of the meat surface. Sixty min of tumbling resulted in a lower concentration of solutes near the surface and a deeper diffusion into the center of the meat. The mechanical effect provided by the strong tumbling resulted in the most profound distribution compared to the soft tumbling. The authors concluded that tumbling is an effective method for solute transfer into the meat. Some studies for quality-enhancing in fish fillets have included the tumbling method. Esaiassen et al. (2005) studied the effect of different solutes added with a tumbler on the yield and sensorial enhancement of frozen-thawed cod (*Gadus morhua*) fillets. The authors reported an increase of 35% in weight after 15 min of tumbling fillets in a ratio of 1:1 (fish: brine) and a higher sensory performance (improved taste, smell, juiciness, whiteness, and glossiness, while reduced old/stale taste and smell) of treated fillets compared to control (not brined). Later, Esaiassen et al. (2008) evaluated the effect of a brine added under the tumbling method on the yield and weight loss of Atlantic cod (*Gadus morhua*) fillets during storage in ice for 15 days. The fillets were tumbled for 3 min in a 1:1 solution, drained, wrapped, and stored. The authors reported a 10% yield increase and lower weight loss during storage than non-brined fillets.

2.4.3.4. Active packaging

Synthetic and natural antioxidants can be added to packaging materials to extend food products' shelf life. The antioxidants migrate to the food to absorb oxidation radicals or to scavenge oxygen and other oxidation promoters such as metals or UV, improving the shelf life of foods (Rangaraj et al., 2021). Barbosa-Pereira et al. (2013) developed antioxidant-active films to increase the shelf life of salmon steaks stored for 21 days at 4˚C. In this study, salmon steaks of 1-1.5 cm thickness were packaged in low-density polyethylene (LDPE) films added with different tocopherol-containing antioxidants. The film containing 5% of an antioxidant blend consisting of 90% of tocopherols (63.3% of gama and beta-tocopherol, 21.1% of delta-tocopherol, and 15.5% of alpha-tocopherol) reported the highest protection against lipid oxidation, reducing oxidation by 40% in salmon muscle compared to control (no antioxidant-film). Torres-Arreola et al. (2007) investigated the effect of polyethylene (PE) packaging material added with BHA on the lipid, and protein degradation of sierra fish (*Scomberomorus sierra*) fillets stored for 120 days at -25˚C. The authors reported a significant reduction of lipid oxidation in samples packaged with PE-BHA compared to control (PE without BHA). The TBARS increased to 4.2 and 11.95 mg malonaldehyde equivalents/kg, and hydroperoxides increased to 7 and 14 meq peroxide/kg in PE-BHA and PE samples, respectively. The protein deterioration was reduced with PE-BHA packaging material compared to control. In recent years, many studies have been performed on the storage of fish by-products and the extraction of bioactive compounds from them. However, to the best of our knowledge, no studies have been conducted to investigate the stabilization of fish by-products through tumbling or dipping to handle and store whole (not ground) pieces of by-products. Furthermore, no studies have separated by-product parts (e.g., reactive organs) to better understand and control oxidative degradation during storage.

CHAPTER 3. MATERIALS AND METHODS

3.1. Experimental Materials

3.1.1. Source of Atlantic salmon by-products

Farmed Atlantic salmon (*Salmo salar*) by-products (whole heads, frames, and viscera containing reactive organs) were collected in Styrofoam boxes on ice from a processing plant in Atlantic Canada The salmon by-products were 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, as described in sections 4.2.1 and 4.2.2 below.

3.1.2. Chemicals

Sodium thiosulfate, sodium hydroxide pellets, isooctane (HPLC grade), glacial acetic acid, potassium iodide, sodium lauryl sulfate, and phenolphthalein (1% alcoholic) were purchased from Fisher Scientific (www.fischersci.ca). Ethanol (95%) was purchased from Commercial Alcohols (www.greenfield.com). Potato starch, *p*-Anisidine reagent, and Alcalase 2.4L were purchased from Sigma-Aldrich (www.sigmaaldrich.com). The antioxidant (ingredients: rosemary extract with 4% carnosic acid and ascorbic acid) was provided by Frutarom (www.iff.com), recommended for its specificity to stabilize highly unsaturated fatty acids present in fish oil and fish materials.

3.2. Experimental Design

Three factors were studied in the present research: reactive organs, processing methods, and antioxidant addition. Firstly, according to the hypothesis that a high load of heme and other reactive compounds increases the lipid oxidation of fish materials during storage, the effect of

reactive organs, and the influence of by-product type (with reactive organs/without reactive organs) on the oxidation rate were studied to propose the handling method for by-product storage. Secondly, with the hypothesis that the grinding process increases the lipid oxidation of fish materials during storage, the effect of the tumbling and grinding process and the influence of the processing method (tumbling/grinding) on the oxidation rate were studied to propose the handling method for by-product storage. Thirdly, the proposed procedures for handling salmon by-products derived from the previous studies were evaluated in freezing storage conditions, including the effect of an antioxidant added through tumbling and grinding. The present research was divided into two storage studies. The first storage study was conducted at 10˚C for 7 days and evaluated the factors: reactive organs and processing methods. The second storage study was conducted at -18˚C for 90 days and evaluated the factor: antioxidant addition.

3.2.1. Storage study 1: Evaluation of the effect of the presence of reactive organs and processing method on lipid oxidation of salmon by-products

In this study, the effect of the presence of reactive organs (gills, heart, liver, bile sac, kidney, spleen, swim bladder, and gonads) on the lipid oxidation of ground heads and viscera was investigated. In addition, the effect of grinding and tumbling processing methods on the lipid oxidation of whole heads, frames, and viscera was also studied. The study was conducted at 10˚C in the absence of light (dark storage) and the presence of oxygen (samples packaged with a partial vacuum). The peroxide, *p*-Anisidine, TOTOX values, and free fatty acid % of the extracted oil were analyzed on days 1 and 7 of storage.

3.2.1.1. Effect of Reactive organs

For the sorting of reactive organs, a first shipment of heads, frames, and viscera were manually separated into their minor components (organs). The sorting of reactive organs was conducted according to the organs containing significant blood, air, and other prooxidant compounds. The reactive organs in heads were determined as gills, while no reactive organs were measured in frames. The reactive organs from all the organs in the viscera were determined as heart, liver, bile sac, kidney, spleen, swim bladder, and gonads.

To analyze the presence of reactive organs on the oxidation rate of by-products, a shipment of heads and viscera was divided into two batches; the reactive organs were removed from one batch of heads and viscera, separately, while the second batch was left whole (containing reactive organs). Afterward, the materials were ground, subjecting them three times to a meat grinder (Model 4146 The Hobart MFG. Co. Ltd.) using discs of 17, 15, and 13 mm to gradually reduce the size until a homogeneous mass of fish material was formed. The ground fish materials were packaged in bags weighing approximately 400 g, with a partial vacuum (Hobart 350 Vacuum packaging machine). The samples were placed in a dark storage refrigerator (Fisher Scientific Isotemp R) at 10˚C, and three samples were removed on days 1 and 7 for oil extraction and oxidation analysis. The parameters studied are shown in Table 3.

3.2.1.2. Effect of Processing methods

For the analysis of processing methods, heads, frames, and viscera were separately divided in two batches. One batch of each by-product was subjected to the grinding process, while the other batch was subjected to the tumbling process.

Table 3. Parameters for the evaluation of the effect of the presence of reactive organs on lipid oxidation of salmon by-products.

Factors	Parameters
Salmon by-product	Head, viscera
By-product type	With reactive organs, without reactive organs
Processing method	Grinding
Sampling	Day 1 and 7
Storage temperature	10˚C

The grinding process was conducted using a meat grinder (Model 4146 The Hobart MFG. Co. Ltd.) and repeated three times using 17, 15, and 13mm discs to gradually reduce the size until a homogeneous mass of fish material was formed. The tumbling process was performed using a tumbler (Vacuum Tumbler VTS-42, BIRO, USA) consisting of a rotatory drum and two internal baffles. The samples were tumbled under a vacuum of 20 mmHg for 15 min in total, including 5 min clockwise, 5 min pause, and 5 min anticlockwise tumbling. The product obtained was an entire piece of the by-product (during tumbling, no loss of shape and size of the material occurs).

Immediately after arrival, half of the salmon by-products were ground, and the other half was tumbled. The ground material, or an entire piece of tumbled material, was packaged in plastic bags, each weighing approximately 400 g, with a partial vacuum (Hobart 350 Vacuum packaging machine). The samples were placed in a dark storage refrigerator (Fisher Scientific Isotemp R) at 10˚C and removed for oil extraction and analysis on days 1 and 7. This study was composed of three treatments: ground by-products with reactive organs (control), ground by-products without reactive organs, and tumbled by-products with reactive organs. The parameters studied are shown in Table 4, and the experimental diagram is illustrated in Figure 4.

3.2.2. Storage study 2: Evaluation of the effect of an antioxidant on lipid oxidation of salmon by-products

Based on the results of storage study 1, tumbled by-products without reactive organs were selected as the control for the second storage study due to the significantly lower lipid oxidation at the 0.05 significance level determined with one-way ANOVA and Tukey's test. The antioxidant used in this study was a commercial antioxidant consisting of a mix of rosemary extract containing 4% carnosic acid and ascorbic acid.

Table 4. Parameters for the evaluation of the effect of processing methods on lipid oxidation of salmon by-products.

Factors	Parameters
Salmon by-product	Head, frames, viscera
By-product type	With reactive organs
Processing method	Grinding, tumbling
Sampling	Day 1 and 7
Storage temperature	10˚C

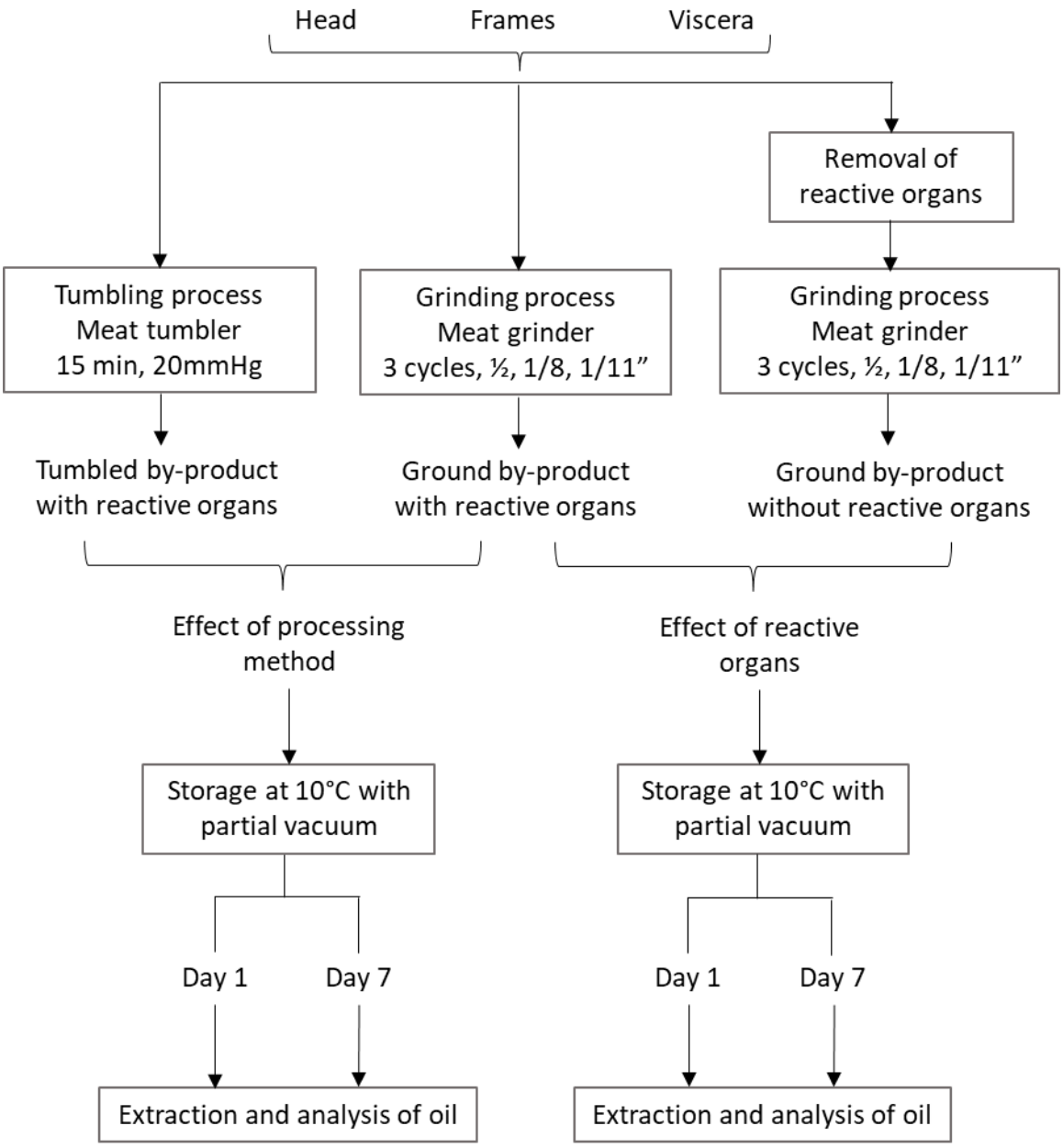


Figure 4. Flow chart showing the experimental design of the storage study at 10˚C for up to 7 days.

Ten grams of the antioxidant was added to 500 mL of distilled water and manually dispersed with a glass spatula. According to the processing method, the antioxidant solution was added to 10 kg of the by-product. 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 progressing to the subsequent grinding cycles. The antioxidant solution was added directly to the tumbler after loading the by-products in the tumbling process. The drum was closed and ran for 15 min with a vacuum of 20 mmHg.

Immediately after the salmon by-products were received, the reactive organs were manually separated from heads and viscera. Each type of by-product was divided into three groups: tumbling without the antioxidant (control), tumbling with the addition of the antioxidant (treatment 1), and grinding with the addition of the antioxidant (treatment 2). An entire piece of each by-product or 400 g of ground product was packaged with a total vacuum (Hobart 350 Vacuum packaging machine) and stored in the dark in a standard industrial freezer room at -18˚C. The peroxide, *p*-Anisidine, TOTOX values, free fatty acid content, and fatty acids profile of the oil extracted were analyzed on days 1, 30, 60, and 90 of storage. The parameters studied are shown in Table 5, and a flowchart of the experimental design is shown in Figure 5.

3.2.3. Enzymatic extraction of fish oil

The method employed for the enzymatic extraction of fish oil was adopted from Dave and Routray (2018) with slight changes, as follows: the frozen fish materials were thawed in cold running water and ground using a Ninja professional blender. A 300 g fish sample was weighted in a 1 L Mason jar. The materials were stirred using a magnetic stirrer, and the pH was adjusted to 8 using 4N sodium hydroxide solution (Hach HQ440d pH meter).

Table 5. Parameters for the evaluation of the effect of added antioxidant (rosemary extract) on lipid oxidation of salmon by-products.

Factors	Parameters
Salmon by-product	Head, frames, viscera
By-product type	Without reactive organs
Antioxidant	Commercial mix (rosemary extract and ascorbic acid)
Antioxidant dosage	0.1%
Processing method	Grinding-Antioxidant, Tumbling-Antioxidant, Tumbling-without antioxidant (control)
Sampling	Day 1, 30, 60 and 90
Storage temperature	-18˚C

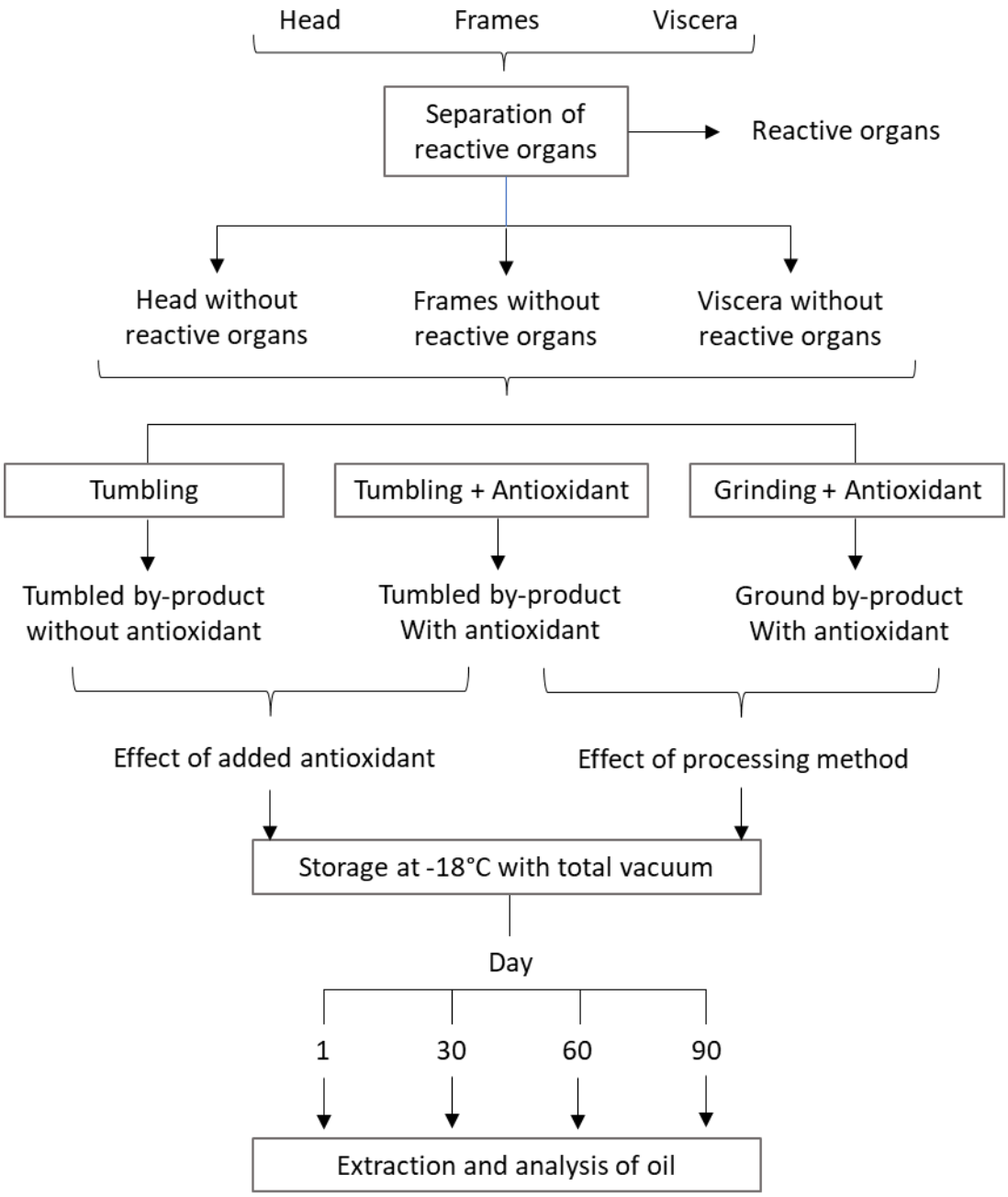


Figure 5. Flowchart showing the experimental design of the storage study at -18˚C for up to 90 days.

Three grams 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 min to deactivate the enzyme. Then the mixture was cooled down to room temperature and centrifuged at 6855 rpm (8000 g) for 10 min (Thermo Scientific LYNX 4000 Centrifuge). The top oil layer was collected and stored with nitrogen in the dark at -80˚C (Panasonic Ultralow Freezer -80˚C) until further analyses. The flowchart of the experimental design of the enzymatic oil extraction from salmon by-products is shown in Figure 6.

3.3. Experimental Analyses

3.3.1. Peroxide value

Peroxide values (PV) were determined following the official method of the American Oil Chemists' Society Cd 8b-90 (AOCS, 1997). A 6 g oil sample was weighed into a 250 mL Erlenmeyer flask and dissolved in 50 mL of 3:2 acetic acid-isooctane solution. 0.5 mL of a saturated solution of potassium iodide was added, and the mixture was allowed to stand for 1 min. Afterward, 30 mL of distilled water, 0.5 mL of 10% sodium lauryl sulfate, and 2 mL of 1% potato starch solution were added. The mixture was swirled and immediately titrated with 0.01 N sodium thiosulfate until the yellow-brownish color disappeared. A blank titration without oil was performed following the same procedure. Peroxide values were determined according to Equation 1:

$$\text{Peroxide value (meq/kg oil)} = \frac{(S-B)\times N\times 1000}{W} \qquad \text{Equation 1}$$

S = Volume of sodium thiosulfate solution for the oil sample titration (mL)

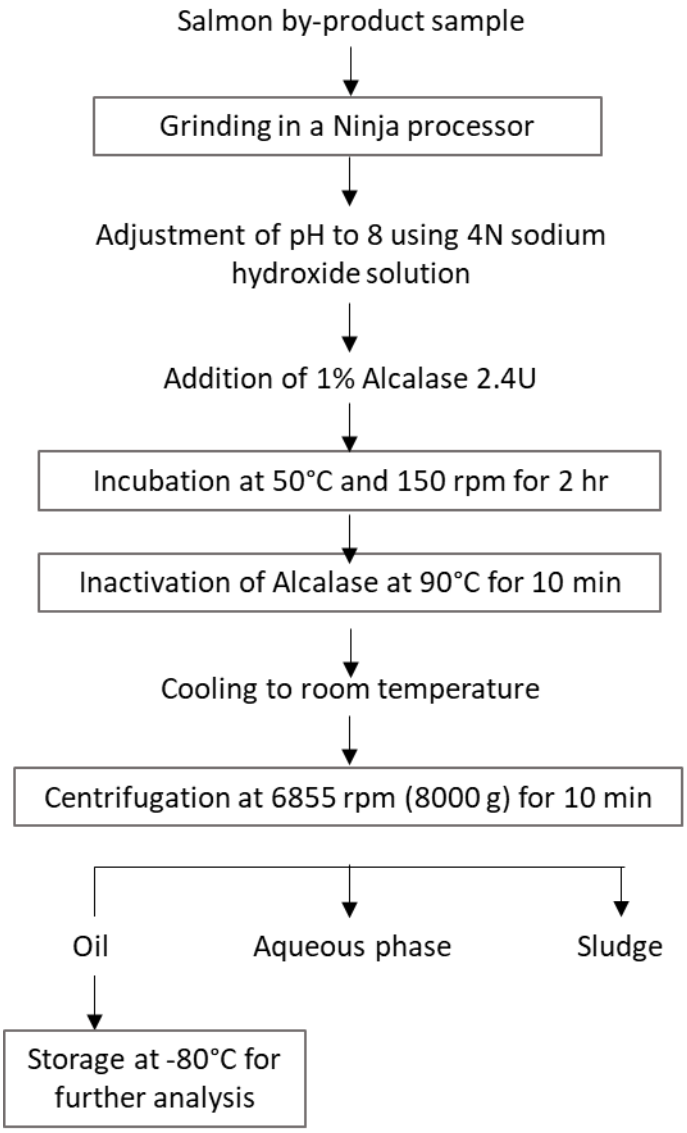


Figure 6. Flowchart showing the experimental design of enzymatic oil extraction from salmon by-products.

B = Volume of sodium thiosulfate solution for the blank titration (mL)

N = Normality of sodium thiosulfate solution (M)

W = Weight of oil (g)

3.3.2. *p*-Anisidine value

p-Anisidine values (AV) were determined following the official method of AOCS Cd 18-90 (AOCS, 1997). A 1 g sample of oil was weighed into a 25 mL volumetric flask. Isooctane was added to dissolve the oil sample and fill the volume to the 25 mL. After thorough mixing, the absorbance of this solution was measured against pure isooctane at 350 nm using a spectrophotometer (DR6000 Spectrophotometer, HACH, USA) and recorded as A_1. Subsequently, 5 mL of this solution was transferred to a 10 mL test tube, and 1 mL of *p*-Anisidine reagent was added. A blank sample was made by mixing 5 mL isooctane with a 1 mL *p*-Anisidine reagent. The solution was homogenized with minimum shaking and left undisturbed for 10 min. Afterward, the absorbance of the solution against the blank was measured at 350 nm and recorded as A_2. *p*-Anisidine values were determined according to Equation 2:

$$p\text{-Anisidine value} = \frac{25\times(1.2A1-A2)}{W} \qquad \text{Equation 2}$$

A1 = Absorbance of the fat solution after reaction with the *p*-Anisidine reagent

A2 = Absorbance of the fat solution

W = Weight of oil (g)

3.3.3. TOTOX value

The TOTOX value is defined as the total oxidation and is calculated using the peroxide and *p*-Anisidine values. TOTOX values were determined according to Equation 3:

TOTOX value = 2PV+AV Equation 3

PV = Peroxide value of the fat solution

AV = *p*-Anisidine value of the fat solution

3.3.4. Free fatty acids contents

Free fatty acid (FFA) content was determined following the official method of AOCS Ca 5A-40 (AOCS, 1997). A sample of 7.05 g oil was weighed into a 250 mL Erlenmeyer flask. Hot neutralized ethanol was prepared by heating 75 mL of 95% ethanol and 2 mL of phenolphthalein to incipient boiling (near 76-77˚C). 77 mL of the hot neutralized ethanol was added to the oil sample. After thorough mixing, the sample was titrated with 0.25 N sodium hydroxide solution until a faint pink color appeared and remained for at least 1 min. FFA (%) were determined according to Equation 4:

$$\text{FFA (\%)} = \frac{\text{mL of alkali} \times \text{N} \times 28.2}{\text{W}} \quad \text{Equation 4}$$

N = Normalityμ of sodium hydroxide solution

W = Weight of oil (g)

3.3.5. Fatty acid profile

The analyses of the fatty acid profile were conducted by the Aquatic Research Center (ARC), Memorial University of Newfoundland, from single samples. This analysis involved lipid

extraction, preparation of fatty acid methyl esters (FAMEs), and fatty acid composition analysis, according to published methods.

3.3.5.1. Lipid extraction

The lipid extraction was performed according to Parrish (1999). An oil sample of 250 µL was weighed in a test tube (previously rinsed with methanol and chloroform) containing 2 mL of chloroform. One milliliter of ice-cold methanol, 1 mL of chloroform: methanol (2:1 v/v), and 0.5 mL of chloroform-extracted water were added to the test tube (Chloroform-extracted water was prepared by adding 1 L of distilled water and 30 mL of chloroform to a separatory funnel. The funnel was manually shaken for 2 min, and the chloroform was removed from the bottom of the funnel. This procedure was repeated twice to remove any lipids present in the distilled water). The test tube was capped and sonicated for 10 min, followed by centrifugation for 3 min at 3000 rpm. The lower organic lipid layer was removed by a double pipetting technique and transferred to a 15 mL lipid-free vial (Parrish & Hooper, 2009). The double pipetting procedure was performed in three steps. Firstly, a 14 cm pipette was passed through the top aqueous layer in the test tube to the bottom by bubbling air with the pipette bulb to prevent the aqueous layer from entering the pipette. Secondly, the pipette bulb was removed, and a 27 cm pipette was placed inside the 14 cm pipette until it reached the bottom of the test tube. Finally, the lipid layer was extracted using the long pipette and transferred to another lipid-free vial. Each short and long pipette was washed with 3 mL ice-cold chloroform, and the wash was collected. The samples were sonicated, centrifuged, double pipetted, and the pipettes were rinsed three times as previously described, and all the organic layers were collected. The collected lipid fraction was evaporated under a gentle stream of nitrogen, sealed with Teflon® tape, and stored at −20 ˚C until further use.

3.3.5.2. Preparation of FAMEs

Forty microliters sample of the lipid extract was transferred to a lipid-free vial containing 1.5 mL of methylene chloride and 3 mL Hilditch reagent. The Hilditch reagent was prepared by adding 1.5 mL of concentrated sulfuric acid to 100 mL of dry methanol. The sample was vortexed for approximately 5 s and sonicated for 4 min. The vial was flushed with nitrogen, capped, sealed with Teflon® tape, and heated at 100 ˚C for 1 h in a VWR drying oven (VWR international, Mississauga, Ontario, Canada). The vial was then cooled to room temperature. Approximately 0.5 mL of a saturated sodium bicarbonate solution in chloroform-extracted water was slowly added to the vial, followed by 1.5 mL of hexane, and vortexed for 10 s. The top organic layer was removed to a new vial, and the hexane was evaporated with a gentle stream of nitrogen. The fatty acids were re-suspended in 0.5 mL of hexane. The vial was sealed with Teflon® tape under nitrogen and sonicated for an additional 4 min.

3.3.5.3. Fatty acid composition analysis

The FAME sample was analyzed on an HP 6890 GC FID equipped with a 7683 autosampler. The GC column was a ZB wax+ (Phenomenex, U.S.A.). The column length was 30m with an internal diameter of 0.32mm. The column temperature was set at 65˚C and held for 0.5 min. The temperature was increased to 195˚C at a rate of 40˚C/min, held 15 min and then ramped to a final value of 220˚C at 2˚C/min. This absolute temperature was maintained for 0.75 min. The carrier gas was hydrogen which was flowed at a rate of 2 mL/min. The injector temperature was set at 150˚C and ramped to a final temperature of 250˚C at a rate of 120˚C/min. The detector temperature stayed constant at 260˚C. Peaks were identified using the retention times of the standards purchased from Supelco, including 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). The chromatograms were integrated using the Agilent OpenLAB Data Analysis - Build 2.203.0.573.

3.3.6. Statistical analysis

All the analyses were conducted in triplicate, except for the analysis of fatty acid profile, which was performed on single sample. The data were analyzed with a one-way analysis of variance (ANOVA) to compare treatments at a 95% confidence level using Minitab 17.3.1. Tukey's tests were performed to compare the quality of the oil obtained from different treatments within and between each time point (except for the fatty acid profile analysis).

CHAPTER 4. RESULTS AND DISCUSSION

4.1. Effect of the presence of reactive organs on lipid oxidation of salmon by-products

4.1.1. Classification and separation of reactive organs

4.1.1.1. Heads

In the present study, the denomination of reactive organs was designated to body parts present in salmon by-products that could fasten lipid oxidation based on their prooxidant nature, such as high acids, salts, and hemoglobin contents, air or oxygen occluded, and thin tissues with high surface contact, which represent a barrier for the storage of by-products. The separation of organs from heads and the grouping of reactive organs were conducted. The heads were separated into gills, eyes, brain, pectoral fins, tongue, cheeks, fatty tissue and cartilage, external facial skeleton, and jaws. Gills were classified as reactive organs from all the organs based on hemoglobin concentration. The organs in salmon heads are illustrated in Figure 7, and the whole salmon head with and without reactive organs are illustrated in Figure 8.

4.1.1.2. Frames

Salmon frames were separated into bones, muscle and spines, skin, dorsal fin, adipose fin, caudal fin, anal fin, and the fragments of kidney remaining internally. In this case, no organs were classified as reactive organs, considering the frames are composed of organs that are potentially minor promoters of lipid oxidation. Therefore, frames were excluded from the study. The organs in a salmon frame are illustrated in Figure 9, and the salmon frame is illustrated in Figure 10.

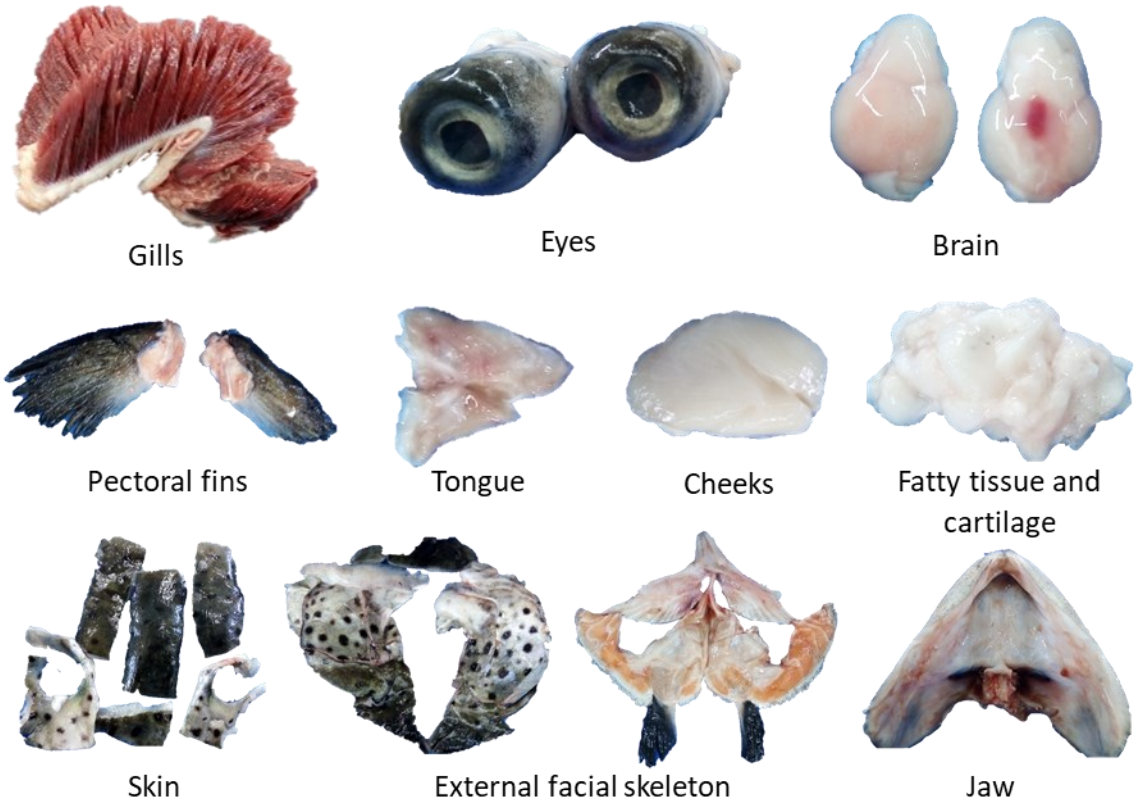


Figure 7. Organs present in salmon head.

Whole salmon head

Salmon head without reactive organs

Figure 8. Whole salmon head with and without reactive organs.

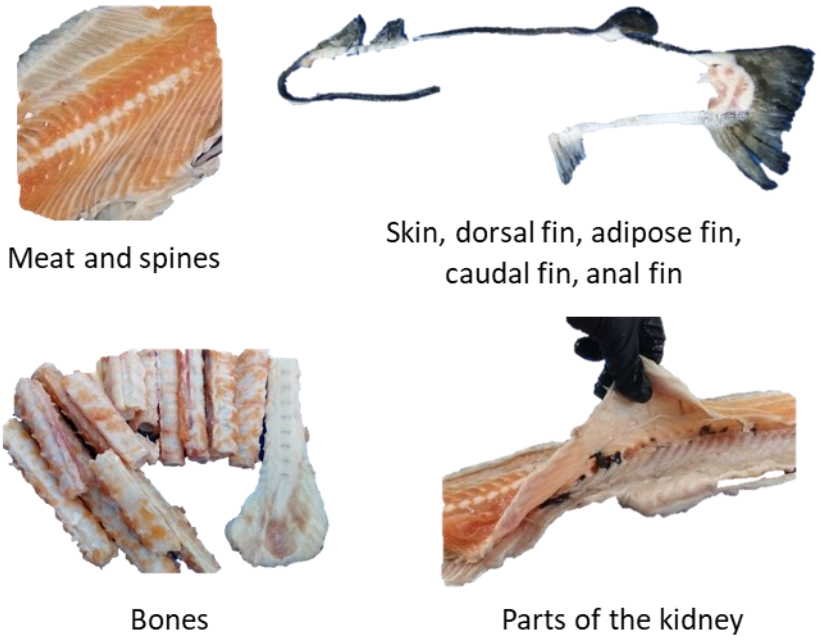


Figure 9. Organs present in salmon frame.

Figure 10. Salmon frame.

4.1.1.3. Viscera

The viscera were separated into heart, liver, bile sac, kidney, spleen, swim bladder, gonads, and the digestive tract consisting of the esophagus, stomach, pyloric caeca, and intestine. The organs classified as reactive organs were the heart, liver, bile sac, kidney, spleen, swim bladder, and gonads. The remaining organs comprised the digestive tract: esophagus, stomach, pyloric caeca, and intestines. The organs in the viscera are shown in Figure 11 (the kidney is not shown), and the whole salmon viscera with and without reactive organs are shown in Figure 12.

4.1.2. Effect of the presence of reactive organs in salmon heads

The quality of the oil extracted from salmon heads, with and without the presence of reactive organs, was analyzed on days 1 and 7 of storage (Table 6). The Tukey's pairwise comparison of the oil quality between treatments and time are presented in Figures 13 to 16.

The oil extracted from salmon heads without reactive organs (gills) present showed lower lipid oxidation than the oil extracted from gill-containing heads in the storage study at 10˚C. The exclusion of gills showed a protective effect on heads against lipid oxidation during storage. The oil extracted from salmon heads without reactive organs (gills) present showed lower lipid oxidation than the oil extracted from gill-containing heads in the storage study at 10˚C. The exclusion of gills showed a protective effect on heads against lipid oxidation during storage.

As indicated in Table 6 and Figure 13, the oil from heads without reactive organs present displayed a slightly lower peroxide value on day 1 than the oil from heads with reactive organs, which was not significant at the 0.05 significance level after 7 days of storage at 10˚C. In contrast, the peroxide value of the oil derived from heads containing reactive organs increased significantly from 0.11

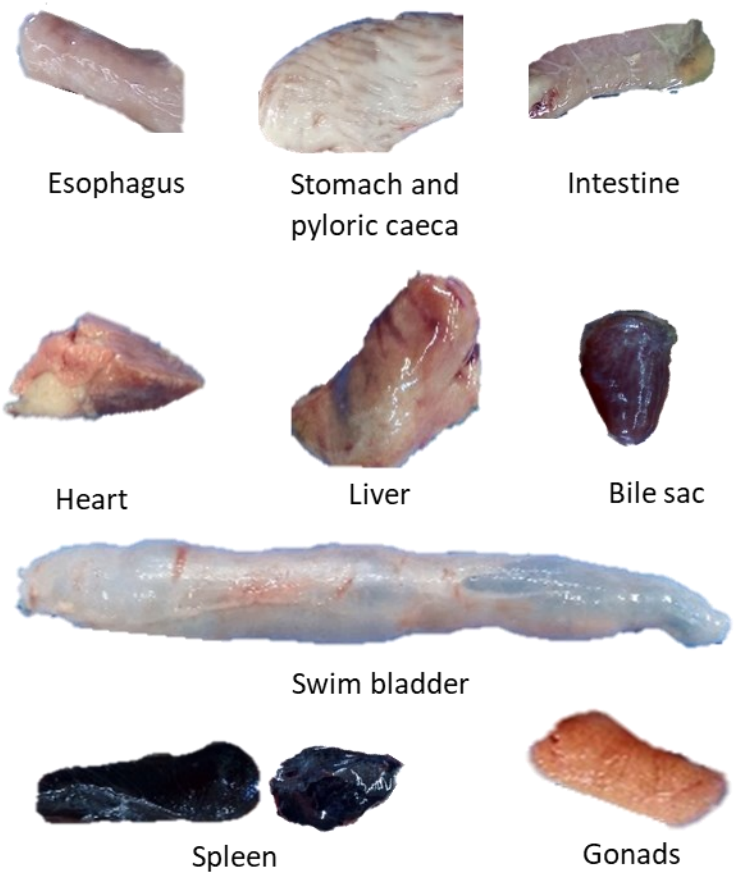
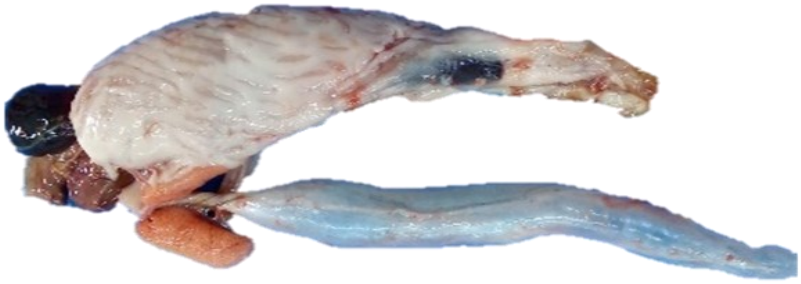
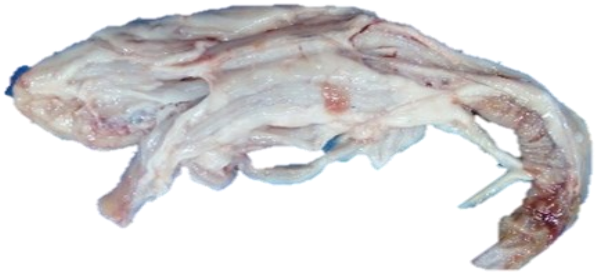


Figure 11. Organs present in salmon viscera.



Whole salmon viscera



Salmon viscera without
reactive organs

Figure 12. Whole salmon viscera with and without reactive organs.

Table 6. Quality analysis of oil extracted from salmon heads with and without the presence of reactive organs stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

Quality analysis	Ground heads with reactive organs		Ground heads without reactive organs	
	Day 1	Day 7	Day 1	Day 7
Peroxide value (meq/kg oil) (± SEM)	0.11 ± 0.05^{b}	0.67 ± 0.03^{a}	0.0 ± 0.0^{b}	0.19 ± 0.0^{b}
p-Anisidine value (± SEM)	1.15 ± 0.25^{a}	1.64 ± 0.28^{a}	1.35 ± 0.05^{a}	1.41 ± 0.28^{a}
TOTOX value	1.37^{b}	2.97^{a}	1.35^{b}	1.80^{b}
Free fatty acid (%) (± SEM)	0.63 ± 0.06^{c}	1.57 ± 0.06^{a}	0.37 ± 0.03^{d}	1.23 ± 0.06^{b}

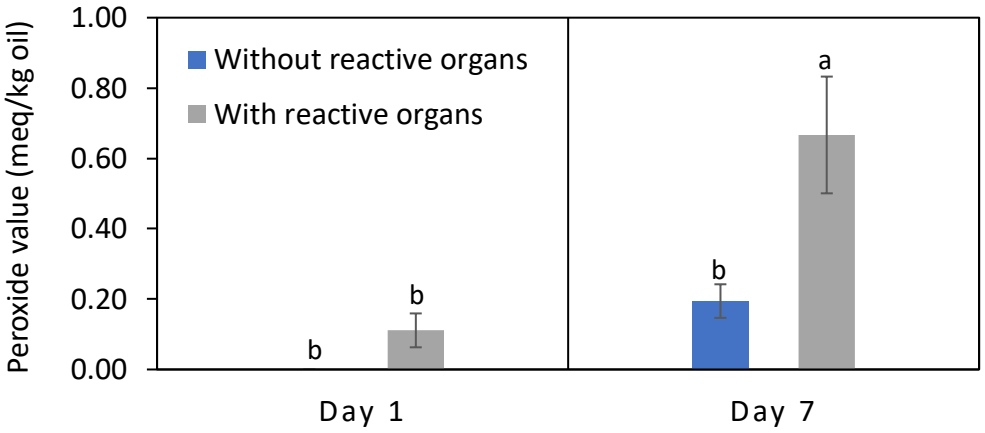


Figure 13. Peroxide values of oil extracted from salmon heads stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

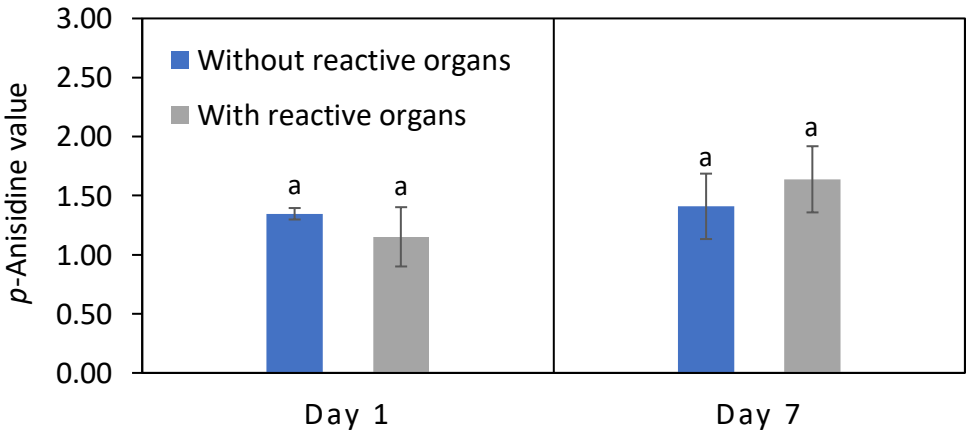


Figure 14. *p*-Anisidine values of oil extracted from salmon heads stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

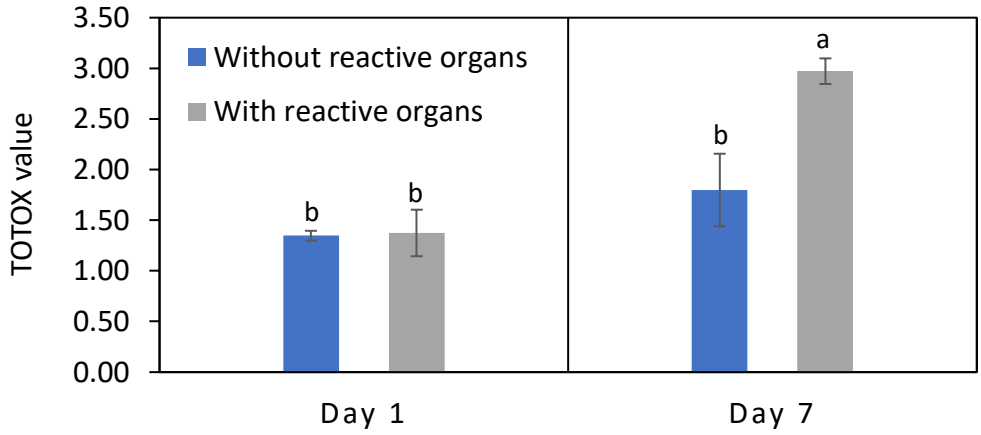


Figure 15. TOTOX values of oil extracted from salmon heads stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

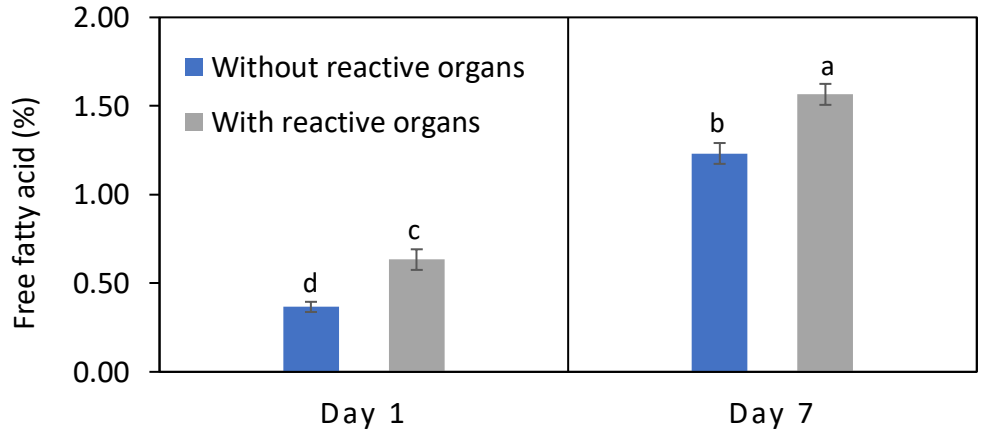


Figure 16. Free fatty acid % of oil extracted from salmon heads stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

on day 1 to 0.67 meq/kg on day 7. Although the peroxide values in the oil of both types of salmon heads were below the permissible limit for human consumption of 5 meq of peroxides/kg oil (Codex Alimentarius Commission, 2017), the reactive organs promoted significant lipid oxidation in salmon heads during storage as determined at the 0.05 significance level. The *p*-Anisidine value did not change significantly during storage, regardless of the presence of reactive organs. The low increase in peroxide and *p*-Anisidine values indicated that the primary oxidation was slow, and the secondary oxidation was not initiated. The TOTOX value showed a significant difference at the 0.05 significance level in the oil from heads with reactive organs present on day 7, where the value was increased 116% (2.97) compared to day 1 (1.37) and 65% compared to the oil from heads without reactive organs present on day 7 (1.80). As shown in Figure 16, the free fatty acid content significantly increased in both treatments at the 0.05 significance level, indicating the activity of lipases in the ground salmon heads regardless of the presence of reactive organs. However, the oil's significantly lower free fatty acid content extracted from heads without gills than heads with gills indicated that excluding reactive organs prevented lipid hydrolysis. Generally, the oil extracted from heads with and without reactive organs showed lipid oxidation and hydrolysis during the seven days of storage at 10˚C. However, the oil from ground heads with reactive organs present had significantly higher lipid oxidation and hydrolysis at the 0.05 significance level than that from heads without reactive organs present. Although the effect of the presence of reactive organs on the lipid oxidation of salmon heads was significant at the 0.05 significance level, the impact on quality was low from a practical point of view, as the oxidation values did not exceed the permissible limits for human consumption (Codex Alimentarius Commission, 2017).

A high storage temperature of 10˚C for fatty fish is expected to promote lipid oxidation, as reported in previous studies (Aidos et al., 2003; Fidalgo et al., 2020; Wu & Bechtel, 2008, 2009).

One possible reason for the low oxidation observed in the present study could be the salmon's lower content of highly PUFAs. While some studies reported quantities of approximately 20 g/100 g oil of total EPA and DHA (Dave et al., 2014; Skåra et al., 2004; Wu & Bechtel, 2008), other recent studies reported amounts of approximately 5 g/100g oil of these fatty acids in salmon by-products (Dave & Routray, 2018; Liu et al., 2020; Routray et al., 2017). In addition, the lipid oxidation process of the oil from salmon heads in the present study may have progressed to the third stage of oxidation which resulted in the low content of primary and secondary oxidation products. Fidalgo et al. (2020) studied vacuum-packaged Atlantic salmon (*Salmo salar*) steaks (60 g weight, 1.5 cm thickness) stored at 10˚C for 15 days and reported a slight decrease in the amounts of primary and secondary oxidation products (as indicated by peroxide value and TBARS) with no statistical difference ($p<0.05$). However, the formation of tertiary oxidation products was significant at the 0.05 significance level and positively correlated with the decrease in EPA and DHA content during storage.

4.1.3. Effect of reactive organs in salmon viscera

The quality of the oil extracted from salmon viscera, with and without the presence of reactive organs, was analyzed on days 1 and 7 of storage (Table 7). The Tukey's pairwise comparisons of the oil quality between treatments and time are presented in Figures 17 to 20.

As indicated in Table 7, the lipid oxidation in the oil extracted from viscera was more significant than in head oils under the same storage conditions. In addition, the effect of the presence of reactive organs on lipid oxidation was more evident in the analysis of the oil extracted from

Table 7. Quality analysis of oil extracted from salmon viscera with and without the presence of reactive organs stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

Quality analysis	Ground viscera with reactive organs		Ground viscera without reactive organs	
	Day 1	Day 7	Day 1	Day 7
Peroxide value (meq/kg oil) (± SEM)	0.25 ± 0.01^{c}	4.89 ± 0.46^{a}	0.06 ± 0.01^{c}	1.39 ± 0.06^{b}
p-Anisidine value (± SEM)	1.05 ± 0.27^{b}	2.21 ± 0.27^{a}	0.47 ± 0.13^{c}	1.12 ± 0.1^{b}
TOTOX value	1.55^{c}	12.00^{a}	0.58^{c}	3.90^{b}
Free fatty acid (%) (± SEM)	4.23 ± 0.38^{c}	12.35 ± 1.06^{a}	3.1 ± 0.3^{c}	8.23 ± 0.51^{b}

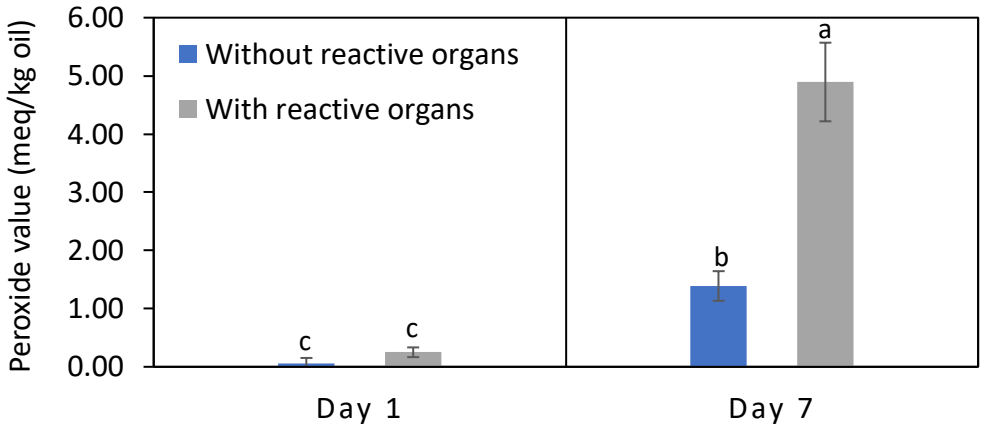


Figure 17. Peroxide values of oil extracted from salmon viscera stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

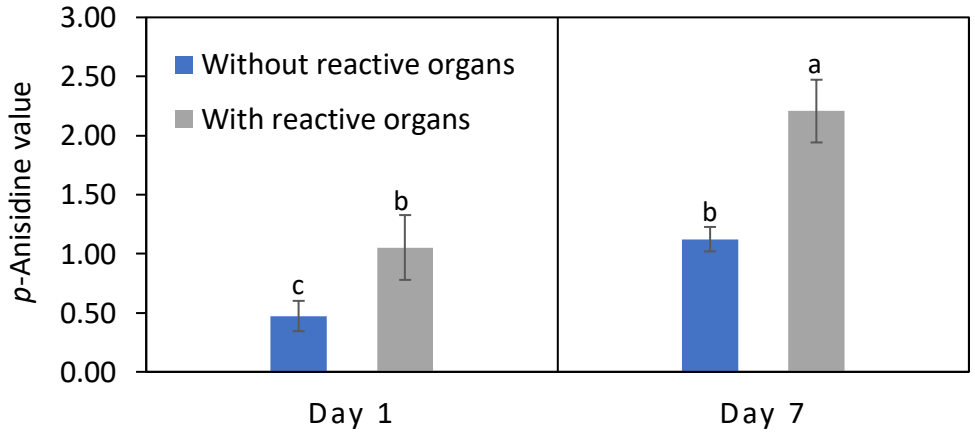


Figure 18. *p*-Anisidine values of oil extracted from salmon viscera stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

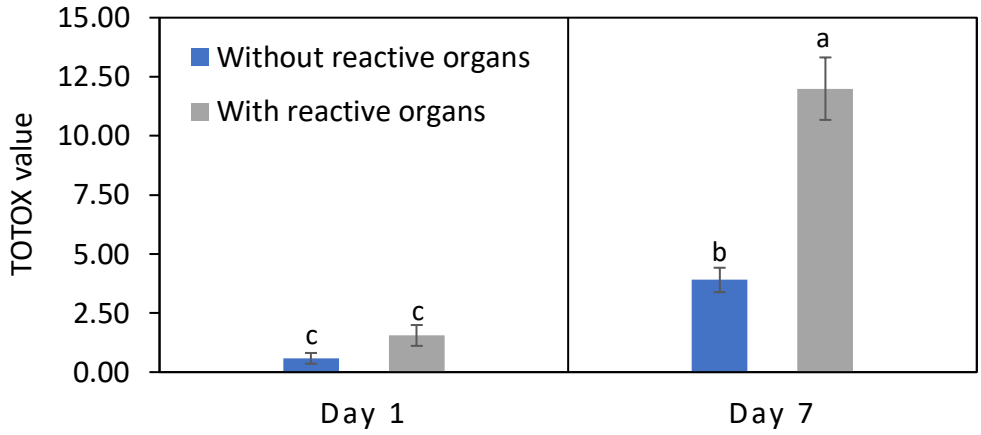


Figure 19. TOTOX values of oil extracted from salmon viscera stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

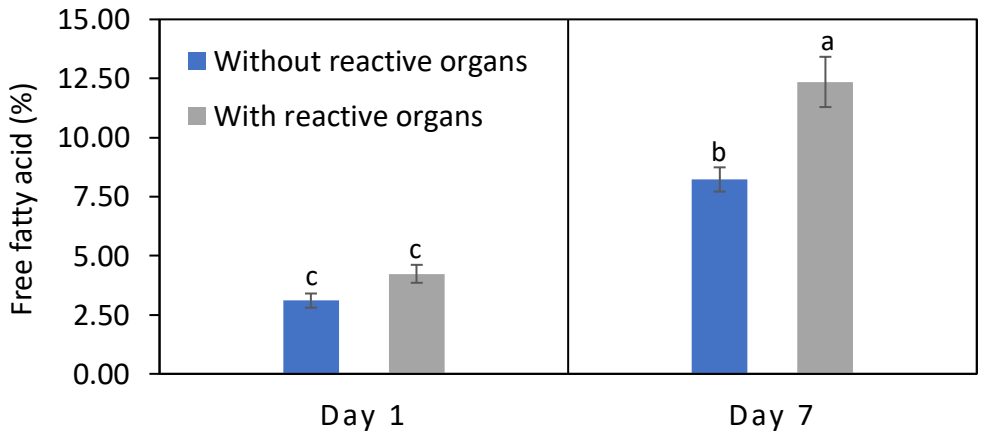


Figure 20. Free fatty acid % of oil extracted from salmon viscera stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

viscera. The peroxide values of the oil from salmon viscera with and without reactive organs present on day 1 were low and approximated those found in salmon heads oils on the same day. However, after 7 days of storage at 10˚C, the lowest value found in the oil from viscera (1.39 meq/kg) was over 2 times the highest value in the oil from heads after storage (0.66 meq/kg) under the same conditions, indicating the higher susceptibility of salmon viscera to lipid oxidation. Furthermore, the peroxide values in the viscera oil with reactive organs present increased to 4.89 meq/kg after storage, approximating the quality limit of 5 meq/kg for human consumption (Codex Alimentarius Commission, 2017). The *p*-Anisidine value significantly increased at the 0.05 significance level with storage time for both treatments and between treatments in both sampling days. The value in the oil from viscera without reactive organs present on day 7 (1.12) was similar to the initial value found in viscera with reactive organs on day 1 (1.05), which further increased to 2.21 after storage. The presence of reactive organs resulted in a 110% increase of the *p*-Anisidine value in the oil extracted from salmon viscera. The high content of peroxides and the increase in *p*-Anisidine values in the viscera oil indicated that the lipid oxidation occurred within the first and second stages of the reaction. In parallel, the TOTOX values of the samples increased from 0.58 and 1.55 on day 1 to 3.9 and 11.96 on day 7 in the oil extracted from viscera without reactive organs and with reactive organs, respectively. The presence of reactive organs promoted the increase of the TOTOX value by 206%.

The content of free fatty acids demonstrated the lipid deterioration in the salmon viscera. While a maximum of 1.56 % of free fatty acids was observed in the oil from heads on day 7, the oil from viscera showed 3.1 and 4.23% free fatty acids, which further increased to 8.22 and 12.35% on day 7 (Table 7), above the maximum allowable limit of 7% for crude fish oil (Bimbo, 1998). The significantly increased free fatty acid content in viscera oil was likely due to the promotion of

lipolysis by digestive enzymes present in the viscera, resulting in the release of high amounts of fatty acids from the viscera lipids (Xu et al., 2019). The content of free fatty acids in the oil extracted from the viscera was significantly lower when the reactive organs were detached from the viscera before storage, indicating the promotion of lipid hydrolysis by the reactive organs.

The quality analysis of the oil extracted from salmon heads and viscera confirmed the promotion of lipid deterioration by the reactive organs. However, viscera were significantly more affected than heads. As stated previously, the high content of free fatty acids will promote lipid oxidation due to the migration of free fatty acids to the aqueous-lipid phase, resulting in the increased exposure of the lipids to oxygen (Liu et al., 2020).

Generally, fish viscera is the most perishable type of by-product due to the diversity of organs and digestive enzymes' load. Therefore, they are more susceptible to lipid oxidation and hydrolysis than other fish parts. Liu et al. (2021) studied the quality of oil enzymatically extracted from salmon (*Salmo salar*) by-products under different operational parameters and reported higher lipid oxidation and hydrolysis in viscera than a mix of heads and frames. Dave et al. (2014) characterized the oil enzymatically extracted from salmon (*Salmo salar*) heads, frames, and viscera using Sea-B-Zyme L200 and reported low lipid oxidation and hydrolysis except in the viscera oil, where the free fatty acids content was 6.49-8.76%. Dave and Manuel (2014) studied crude salmon (*Salmo salar*) oil extracted from viscera using a scraped-surface heat exchanger and with the addition of an antioxidant and reported high oxidation (peroxide value of 9.17 meq/kg, *p*-Anisidine value of 3.36, and TOTOX value of 21.69), and low free fatty acid content (1.23%). A high level of lipid oxidation may have resulted from the high content of PUFAs and heat applied during the oil extraction (Dave et al., 2014), and the level of lipid hydrolysis can be affected by the type of

enzyme used for oil extraction and acid/alkaline environment employed for the appropriate enzyme activity (Liu et al., 2021).

Hwang et al. (2004) compared the lipid quality of the oil extracted from minced muscle and a mix of muscle and viscera (including liver, digestive tract, bile sac, and storage fat) of farmed Korean catfish (*Silurus asotus*). The raw material was stored in a dark place and exposed to air for 12 days at 2˚C. The oil from the mix of muscle and viscera showed significantly higher lipid oxidation and lipolysis at the 0.05 significance level than the oil from muscle, as indicated by the PV, TBARS, and FFA contents (an approximate value of 2 meq/kg, 1.5 mmol MA/g, and 28 mmol/g of PV, TBARS, and FFA, and 1 meq/kg, 0.8 mmol MDA/g, and 10 mmol/g of PV, TBARS, and FFA in the mix of muscle and viscera oil and in the muscle oil, respectively). The higher oxidation found in the oil from the mix of muscle and viscera was related to a high content of PUFAs of the lipids (3-4.1% of EPA and 5.4-10.8% of DHA in mix of muscle and viscera oil, and 2.7-3.7 of EPA and 4.4-9.4 of DHA in muscle oil), and the higher lipid hydrolysis at the 0.05 significance level was related to the abundance of lipolytic enzymes in the viscera. Similarly to the present study, the presence of viscera organs promoted significantly higher oxidation (at the 0.05 significance level) on the lipids of a fatty fish during storage compared to the fish muscle.

In contrast, Wu et al. (2022) reported lower lipid oxidation obtained in minced herring (*Clupea harengus*) viscera and belly flap than heads, frames, tail, and fillet. The fish material was stored and exposed to air for 4 days on ice. The oil from the mix of viscera and belly flaps showed significantly lower oxidation, while the oil from heads showed significantly higher oxidation (at the 0.05 significance level), as indicated by the PV and TBARS contents (an approximate of 300 µmol/kg and 75 µmol/kg of peroxide and TBARS, and 900 µmol/kg and 225 µmol/kg of peroxide and TBARS in viscera and felly flap oil, and heads oil, respectively). The lower lipid oxidation

obtained in the viscera and belly flaps oil was related to a significantly higher content of α-tocopherol (near 18 mg/kg) (at the 0.05 significance level) compared to heads, frames, tails, and fillet. The higher lipid oxidation obtained in the heads oil was related to a significantly higher content of hemoglobin (near 100 µmol/kg) and lipoxygenase activity (near 1.60), while an absence of α-tocopherol was observed in the samples. No significant correlation was found between lipid oxidation with the lipid content and the total PUFA content. These results indicated the impact of type and amount of prooxidants on the lipid oxidation of fatty fish by-products rather than lipid and PUFA content.

4.2. Effects of processing methods on lipid oxidation of salmon by-products

4.2.1. Effects of processing methods on salmon heads

The quality of the oil extracted from tumbled and ground heads was analyzed on days 1 and 7 of storage (Table 8). The Tukey's pairwise comparisons of the oil quality between treatments and time are presented in Figures 21 to 24.

The effect of high processing (grinding) was compared to that of low processing (tumbling) on the lipid oxidation of salmon heads during seven days of storage at 10˚C. The peroxide values in the oil extracted from tumbled and ground heads on day 1 were close to zero and not significant at the 0.05 significance level. Peroxides were formed during the storage of ground heads, increasing from 0.11 to 0.67 meq/kg between days 1 and 7. In comparison, the content of peroxides in the oil extracted from tumbled heads did not increase from day 1 to day 7, suggesting no development of the first stage of lipid oxidation in heads processed using this method. The grinding method promoted oxidation, while tumbling showed no effect on salmon heads' lipid oxidation under the storage conditions evaluated during the present study.

Table 8. Quality analysis of oil extracted from tumbled and ground heads stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

Quality analysis	Ground heads		Tumbled heads	
	Day 1	Day 7	Day 1	Day 7
Peroxide value (meq/kg oil) (± SEM)	0.11 ± 0.05^{b}	0.67 ± 0.03^{a}	0.11 ± 0.05^{b}	0.06 ± 0.01^{b}
p-Anisidine value (± SEM)	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 (%) (± SEM)	0.63 ± 0.06^{c}	1.57 ± 0.06^{a}	0.33 ± 0.06^{d}	0.92 ± 0.1^{b}

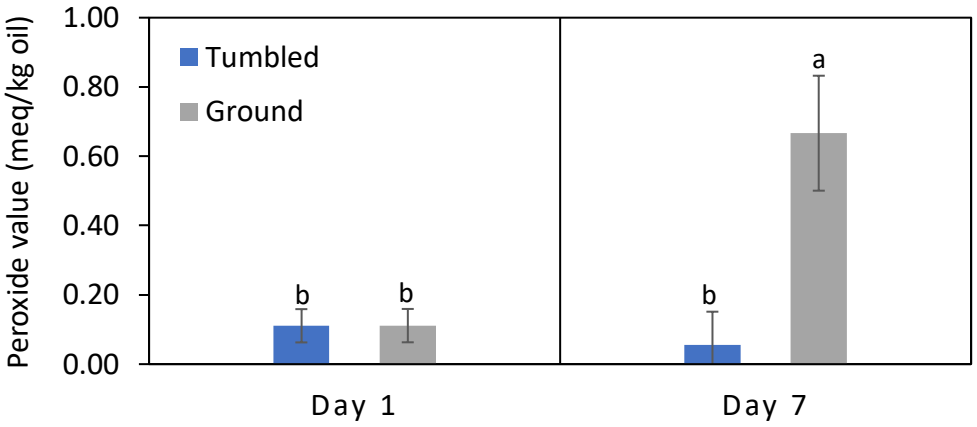


Figure 21. Peroxide values of oil extracted from salmon heads stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

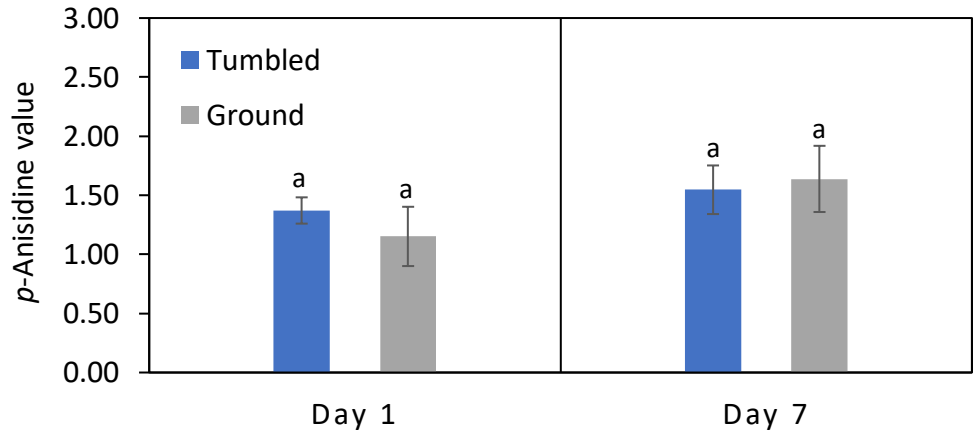


Figure 22. *p*-Anisidine values of oil extracted from salmon heads stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

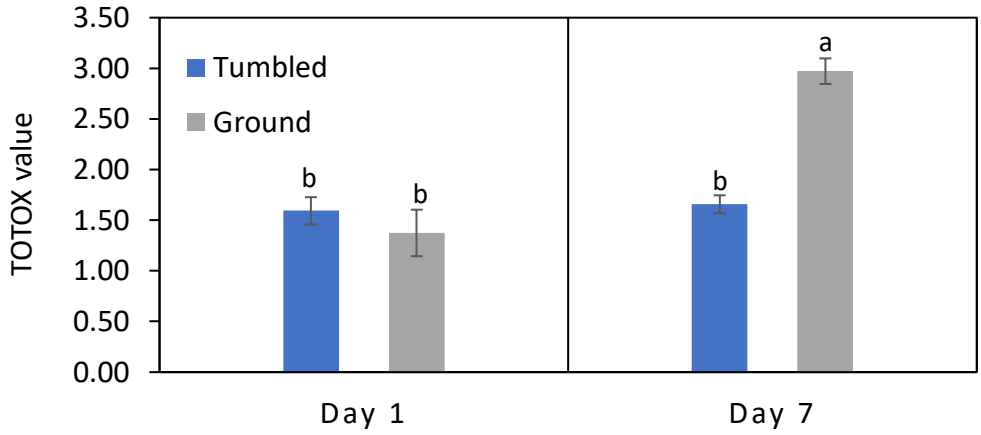


Figure 23. TOTOX values of oil extracted from salmon heads stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

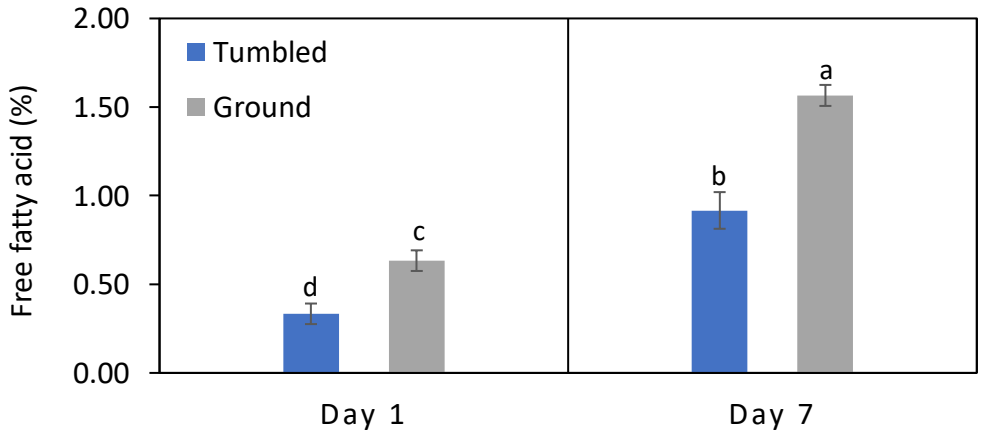


Figure 24. Free fatty acid % of oil extracted from salmon heads stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

The *p*-Anisidine values ranged from 1.15 to 1.64 in all samples and were insignificant at the 0.05 significance level, which suggested no development of the second stage of lipid oxidation in tumbled or ground heads during storage. The oil extracted from tumbled heads exhibited no increase in TOTOX value after storage. In contrast, the oil extracted from ground heads increased 116% after the storage of the heads. The increase in the TOTOX value of the oil extracted from ground heads was due to the increase in the peroxide value of the same samples during storage. Although values below the quality limits of peroxides, *p*-Anisidine, and TOTOX were observed, the oil extracted from ground heads developed significant lipid oxidation at the 0.05 significance level. In comparison, the oil extracted from tumbled heads did not develop lipid oxidation during storage. Therefore, the lower level of processing showed a positive effect on the lipid quality of salmon heads during storage at 10˚C over a period of seven days. As indicated in Table 8 and Figure 24, the formation of free fatty acids was observed in all the treatments, indicating the activity of endogenous enzymes regardless of the processing method employed. However, after seven days of storage of the heads, the free fatty acid content in the oil extracted from tumbled heads was significantly lower at the 0.05 significance level than in the oil extracted from the ground heads (0.92 and 1.57%, respectively).

The highest lipid hydrolysis and oxidation were observed in the oil extracted from ground heads, suggesting that the grinding process promoted lipid deterioration possibly due to increased molecular oxygen from the environment and the access of pro-oxidants to fish lipids during the grinding process. It has been hypothesized that the mincing process promotes lipid oxidation due to the cellular disruption and activation of starters of oxidation such as lipoxygenase (Albertos et al., 2015).

Aidos et al. (2003) 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. The initial peroxide and *p*-Anisidine values in the herring by-products were 4.4 meq/kg and 2.6, respectively, higher than the values observed in salmon by-products in the present study. The higher oxidation was probably due to the extraction method, as the heat method has been reported to promote oxidation during the oil extraction process. Dave et al. (2014) compared the effect of heat and enzymatic methods on the lipid oxidation of oils extracted from salmon by-products. The heat method promoted higher total oxidation values (10.73, 1, and 6.11) than the enzymatic method (0.71, 0.73, and 3.93) in the oil from heads, frames, and viscera, respectively, although the total oxidation of the oil extracted from salmon frames employing both methods was similar. In contrast, Skara et al. (2004) reported total oxidation of 2.3 in the oil extracted by heat method from salmon (*Salmo salar*) heads, frames, and downgraded-gutted fish. The low content of free fatty acids reported (0.2%) was similar to the content found in the oil extracted from heads and frames (< 0.5%) in the present study.

4.2.2. Effects of processing methods on salmon frames

The quality of the oil extracted from tumbled and ground frames was analyzed on days 1 and 7 of storage (Table 9). The Tukey's pairwise comparisons of the oil quality between treatments and time are presented in Figures 25 to 28. Salmon frames had a low level of lipid oxidation, in general, during this storage study. The initial peroxide values in the oil extracted from tumbled and ground frames were significantly different at the 0.05 significance level (0.11 and 0.42 meq/kg, respectively). However, the peroxide value did not change after the storage in any of the treatments. A final peroxide value of 0.14 and 0.42 meq/kg was found in the oils extracted from the tumbled and ground frames, respectively.

Table 9. Quality analysis of oil extracted from tumbled and ground frames stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

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

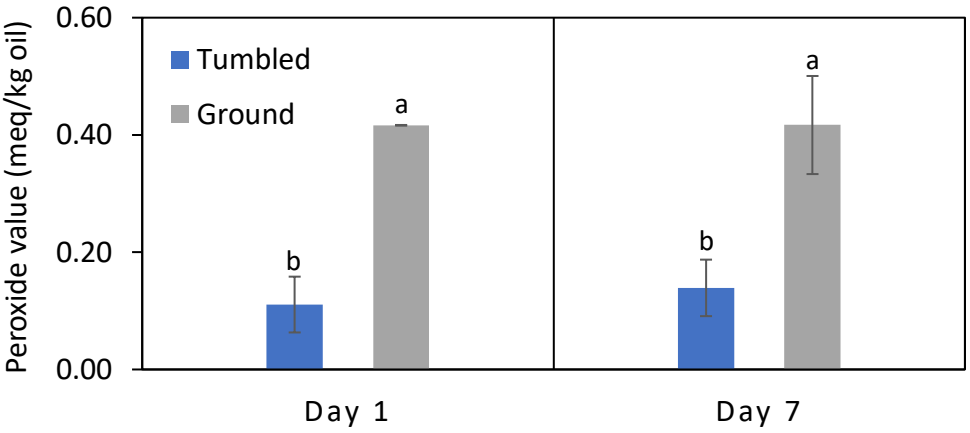


Figure 25. Peroxide values of oil extracted from salmon frames stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

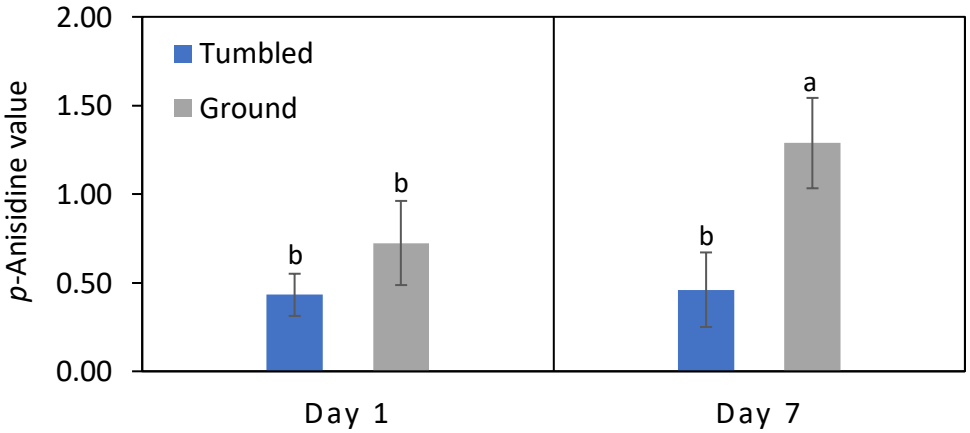


Figure 26. *p*-Anisidine values of oil extracted from salmon frames stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

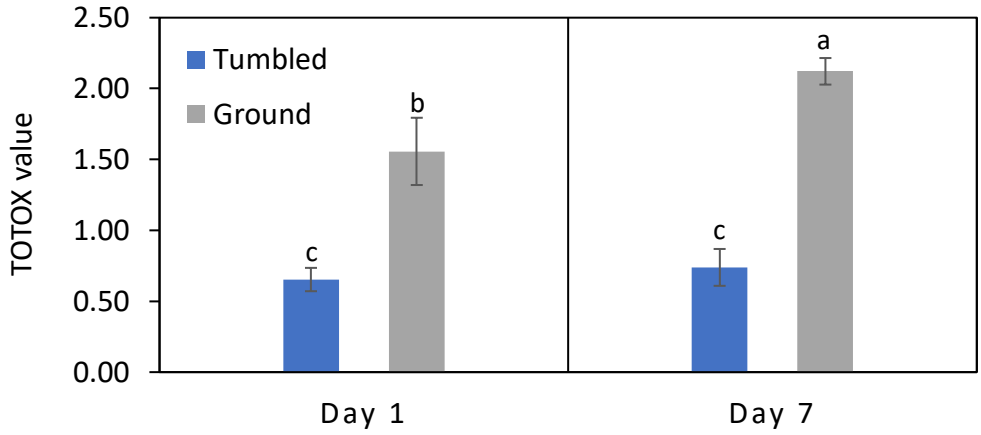


Figure 27. TOTOX values of oil extracted from salmon frames stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

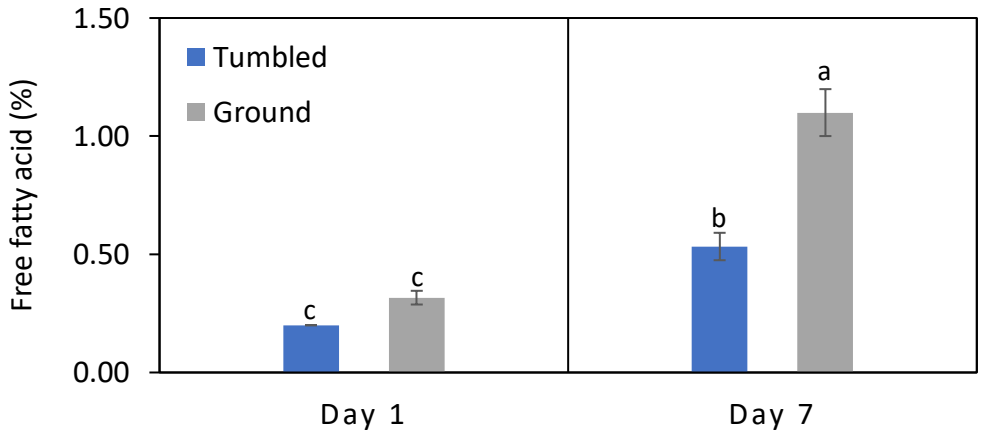


Figure 28. Free fatty acid % of oil extracted from salmon frames stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

The processing method could have promoted an increased peroxide value in the oil extracted from the ground frames compared to that extracted from the tumbled frames. Similar to peroxides, the *p*-Anisidine values in the oil extracted from the ground frames were higher than those of the tumbled frame. The *p*-Anisidine values in the oil extracted from tumbled frames were 0.43 and 0.46 on days 1 and 7, respectively. In comparison, the oil from ground frames increased from 0.72 to 1.29 during the same time. These results indicated the progression of 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). The insignificant increase in the peroxide value of the oil extracted from the ground frames was possibly due to an initial increase and subsequent decrease of the content of peroxides as the lipid oxidation proceeded between days 1 to 7. Overall, the total oxidation value in oil extracted from the tumbled frames increased from 0.65 to 0.74 (which was insignificant at the 0.05 significance level).

Contrastingly, the total oxidation value in the oil extracted from the ground frames increased from 1.56 to 2.12 during storage between storage times and between treatments, which was significant at the 0.05 significance level. Similarly, Wu et al. (2021) reported low initial oxidation in minced salmon (*Salmo salar*) backbones and a statistically significant increase after 11 days of ice storage, as determined by primary and secondary oxidation products (peroxide value and TBARS).

The free fatty acid content increased with the level of processing and with storage time. The free fatty acids increased significantly at the 0.05 significance level from 0.2% to 0.53% and from 0.32% to 1.10% during storage in the oil extracted from the tumbled and ground frames. The free fatty acids increase was likely due to enzymes potentially present in skin and muscle (Kurtovic et al., 2009).

The grinding method promoted the lipid deterioration observed in the ground frames, increasing the TOTOX value and free fatty acid content in the oil extracted from ground frames by 186% and 107% compared to the oil extracted from tumbled frames. Dave et al. (2014) studied the quality of the oil extracted from farmed Atlantic salmon (*Salmo salar*) frames by heat and enzymatic methods and reported low total oxidation under both methods (1 and 0.73, respectively). The oil extracted from salmon frames appeared to be more stable towards heat-induced oxidation, as the heat extraction did not significantly increase the lipid oxidation. Similar to the present study, the lipid oxidation reported in the oil from frames was low.

4.2.3. Effects of processing methods in salmon viscera

The quality of the oil extracted from tumbled and ground viscera was analyzed on days 1 and 7 of storage (Table 10). The Tukey's pairwise comparisons of the oil quality between treatments and time are presented in Figures 29 to 32.

The initial peroxide values in the oil extracted from tumbled and ground viscera were close to zero and increased to 2.17 and 4.89, respectively, after the storage. The peroxide value in the oil extracted from the ground viscera was significantly higher at the 0.05 significance level than the oil extracted from the tumbled viscera by 125%. The oil quality of the ground viscera significantly deteriorated during the storage of the viscera at 10˚C, which was close to the consumption-permissible limit of 5 meq/kg oil (Codex Alimentarius Commission, 2017). The *p*-Anisidine values significantly increased at the 0.05 significance level from 0.87 to 1.54 and from 1.05 to 2.21 in tumbled and ground viscera oils, respectively, indicating the progress of secondary oxidation. The *p*-Anisidine value of the oil from ground viscera was significantly higher at the 0.05 significance level than the oil from tumbled viscera after storage.

Table 10. Quality analysis of oil extracted from tumbled and ground viscera stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

Quality analysis	Ground viscera		Tumbled viscera	
	Day 1	Day 7	Day 1	Day 7
Peroxide value (meq/kg oil) (± SEM)	0.25 ± 0.01^{c}	4.89 ± 0.46^{a}	0.08 ± 0.01^{c}	2.17 ± 0.25^{b}
p-Anisidine value (± SEM)	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 (%) (± SEM)	4.23 ± 0.38^{c}	12.35 ± 1.06^{a}	1.60 ± 0.3^{d}	6.37 ± 0.51^{b}

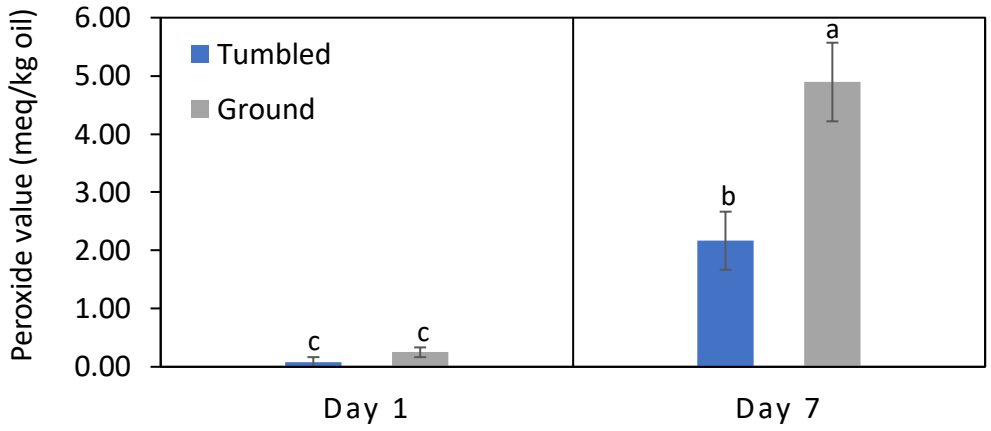


Figure 29. Peroxide values of oil extracted from salmon viscera stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

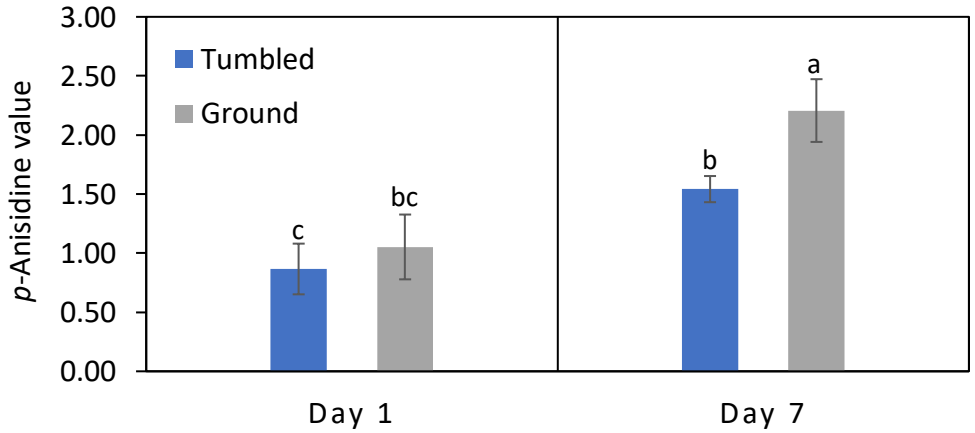


Figure 30. *p*-Anisidine values of oil extracted from salmon viscera stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

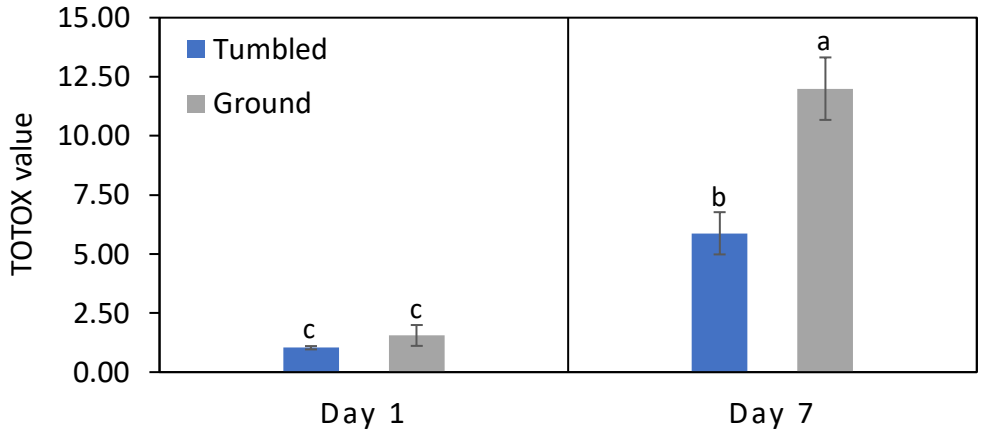


Figure 31. TOTOX values of oil extracted from salmon viscera stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

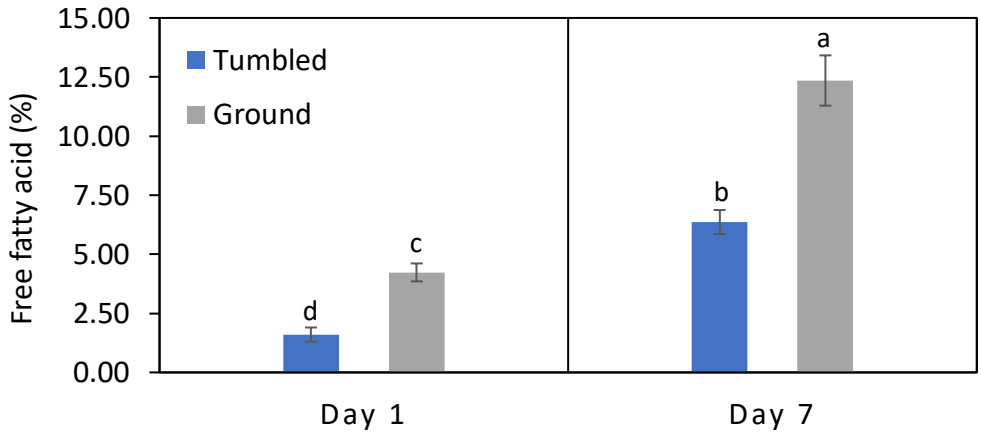


Figure 32. Free fatty acid % of oil extracted from salmon viscera stored at 10˚C for 7 days. The means showing different letters are statistically different ($p<0.05$).

The TOTOX value of the oil from ground viscera (11.96) was significantly higher at the 0.05 significance level than tumbled viscera (5.88) after storage. Therefore, lipid oxidation was more promoted by the grinding process than the tumbling process.

Further, the lipid hydrolysis in the viscera was significant and was affected by the processing method. The content of free fatty acids in the tumbled viscera oil increased from 1.60 to 6.37% during storage, while the content in the ground viscera oil increased from 4.23 to 12.35%. The amount of free fatty acids in the ground viscera oil was significantly higher than tumbled viscera oil, indicating a sound effect of the grinding process on the hydrolysis of viscera lipids. Generally, the quality of the viscera oil significantly deteriorated during storage, and the grinding process further promoted oxidation and hydrolysis.

Wu and Bechtel (2008) determined the quality of the oil extracted from Alaska pink salmon (*Oncorhynchus gorbuscha*) by-products stored for 4 days at 6 and 15˚C. The by-products were stored unprocessed, and the oil was extracted using the heat method. An initial free fatty acid content of 1% was reported, increasing 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. Contrarily, in another study with walleye pollock (*Theragra chalcogramma*), Wu and Bechtel (2009) analyzed the quality of the oil extracted by heat from a mix of heads, frames, skin, and viscera, reporting higher initial oxidation (peroxide value of nearly 10 meq/kg oil and TBARS of 20 µg MDA/g oil) than the present study, probably due to a high level of highly unsaturated fatty acids (EPA and DHA of 20 g/100g oil) and the method of extraction. While high temperature used for oil extraction is related to higher lipid oxidation (Dave et al., 2014), the level of EPA and DHA found in the fish oil is related to the level of marine fats contained in the fish diet (Dave,

2016; Honold et al., 2016). To date, no studies have been performed related to the effect of the processing method on the lipid oxidation of fish by-products during storage.

4.3. Effect of antioxidant addition on lipid oxidation of salmon by-products

4.3.1. Effect of antioxidant addition on salmon heads

The quality of the oil extracted from salmon heads, with and without the addition of the antioxidant, was analyzed on days 1, 30, 60, and 90 of storage (Table 11). The Tukey's pairwise comparisons of the oil quality between treatments and time are presented in Figures 33 to 36.

The present study showed that the grinding method had a more significant effect on promoting oxidation than the addition of an antioxidant in preventing it during the storage of salmon heads at -18˚C over the total 90 days.

During the frozen storage study, tumbled heads without the presence of reactive organs (with and without antioxidant addition) and ground heads without the presence of reactive organs (with antioxidant added) were analyzed. Although the previous section of this study (Section 5.2) reported the promotion of oxidation by the grinding process, grinding was included in the present section to analyze the efficiency of the grinding process in comparison to the tumbling process for adding antioxidants to the salmon by-products.

While the peroxide value exhibited a similar pattern to the oil extracted from tumbled heads regardless of the antioxidant addition, a peak peroxide value was observed in the oil extracted from ground heads which was significantly higher at the 0.05 significance level on day 30. However, the peroxide value observed in all the treatments during the study was under the quality limit for 5 meq/kg of oil (Codex Alimentarius Commission, 2017).

Table 11. Quality analysis of oil extracted from salmon heads, with and without antioxidant (AOX), stored at -18˚C for up to 90 days. The means showing different letters are statistically different ($p<0.05$).

Treatment	Peroxide value (meq/kg oil) (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled heads	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 (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled heads	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 (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled heads	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 (%) (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled heads	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}

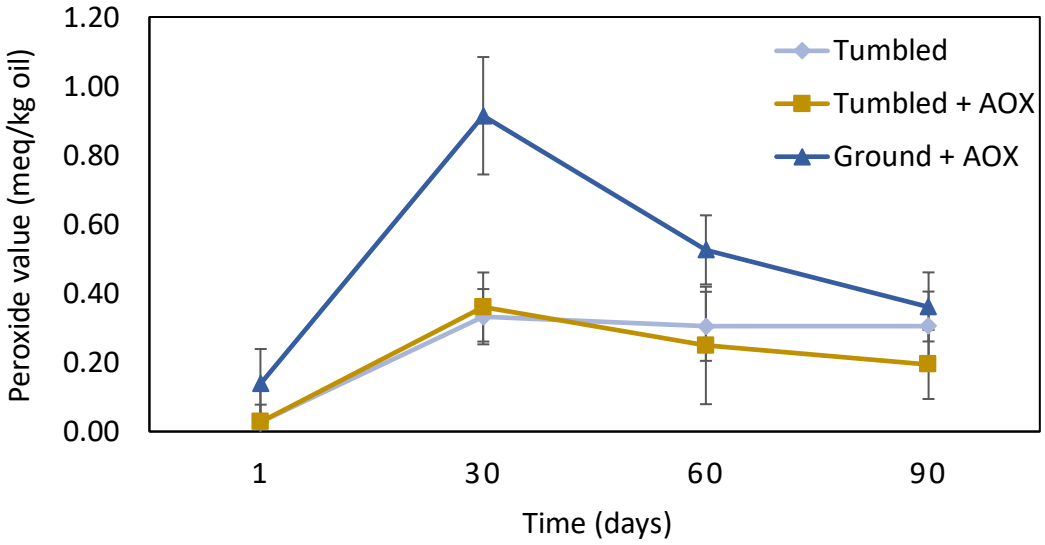


Figure 33. Peroxide values of oil extracted from salmon heads stored at -18˚C for up to 90 days. Values represent mean ±SEM.

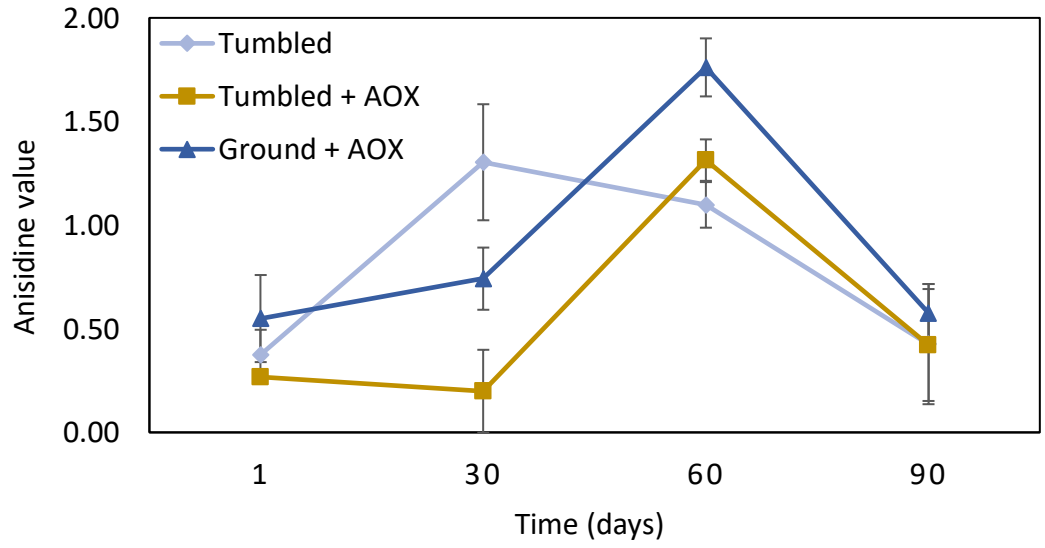


Figure 34. *p*-Anisidine values of oil extracted from salmon heads stored at -18˚C for up to 90 days. Values represent mean ±SEM.

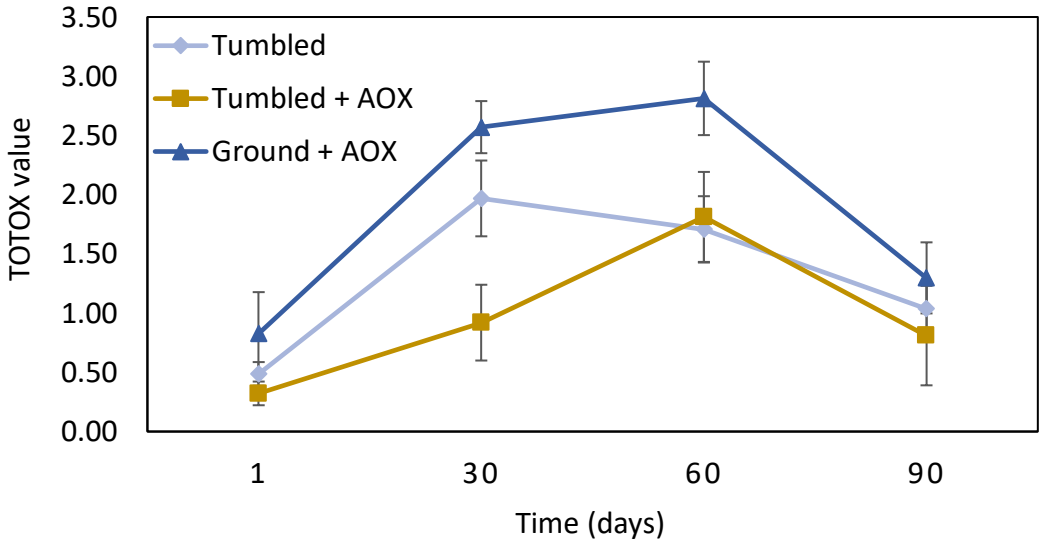


Figure 35. TOTOX values of oil extracted from salmon heads stored at -18˚C for up to 90 days. Values represent mean ±SEM.

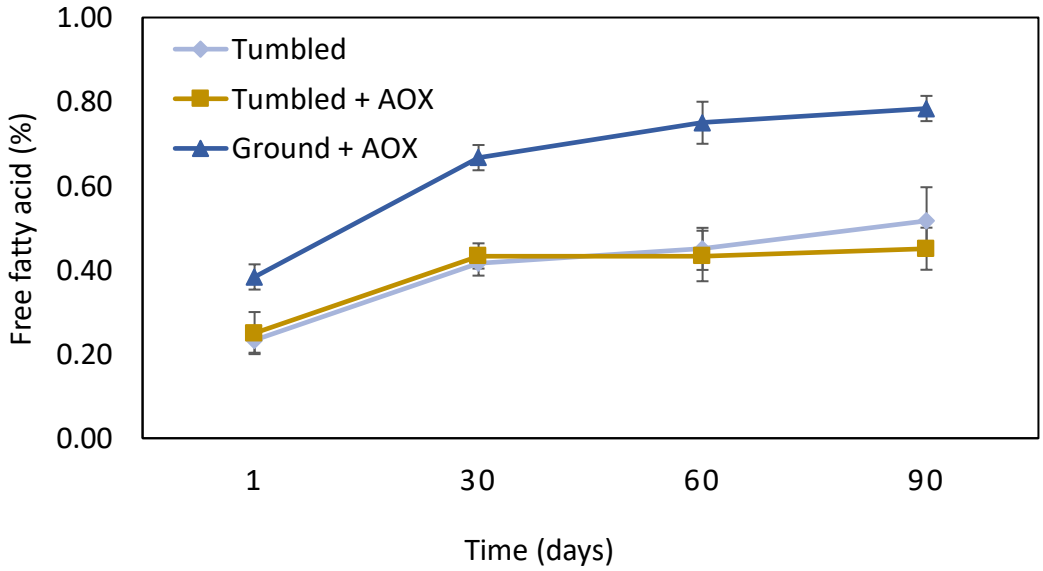


Figure 36. Free fatty acid % of the oil extracted from salmon heads stored at -18˚C for up to 90 days. Values represent mean ±SEM.

The addition of antioxidants to tumbled heads without the presence of reactive organs did not decrease the peroxide value during storage, probably due to the fact that the level of peroxide value in which the samples ranged during the storage was very low (<0.5 meq/ Kg oil). Contrastingly, the grinding method promoted the oxidation of salmon heads lipids with the added antioxidant. The potential positive effect of an antioxidant addition to the heads was surpassed by the negative effect of the grinding method towards lipid oxidation.

A protective effect of adding the antioxidant on lipid oxidation was observed during the analysis of *p*-Anisidine values. The tumbled heads without the antioxidant showed an early increase of *p*-Anisidine value significantly higher at the 0.05 significance level on day 30, after which the value decreased, suggesting the progression of lipid oxidation. The analysis of the oil extracted from tumbled and ground heads with the antioxidant added showed a delayed increase of *p*-Anisidine during the first 30 days of storage compared to the oil from tumbled heads without added antioxidant, suggesting a prevention effect of the antioxidant on the lipid oxidation of heads lipids. The *p*-Anisidine values of the oil from tumbled, and ground heads with the added antioxidant reached the maximum value on day 60 and decreased with further storage, which was likely due to the progression of lipid oxidation. Although not significant at the 0.05 significance level, *p*-Anisidine values of the oil derived from the ground head with added antioxidant were higher than those from the tumbled head with antioxidant.

Similarly, the total oxidation value was higher in the oil extracted from ground heads added with the added antioxidant than the oil from tumbled heads with and without added antioxidant, which was significantly higher at the 0.05 significance level on day 60. In contrast, a lower total oxidation value was recorded in the oil extracted from tumbled heads with added antioxidant, and this was significantly lower at the 0.05 significance level on day 30, indicating the protective effect of the

antioxidant on lipid oxidation. These results indicated the grinding method's promoting effect and the added antioxidant's protective effect on the lipid oxidation of salmon heads during frozen storage. However, any of these treatments were below the quality limits for peroxide, *p*-Anisidine, and TOTOX values of the oils for human consumption (Codex Alimentarius Commission, 2017).

The free fatty acid analysis of the oil extracted from salmon heads indicated the ongoing activity of endogenous enzymes, which promoted the hydrolysis of lipids. Oil extracted from ground heads displayed higher free fatty acid content, which was significantly higher at the 0.05 significance level on days 1, 30, 60, and 90. The lower content of free fatty acids in the oil extracted from tumbled heads compared to that extracted from ground heads confirmed the impact of the grinding method on the quality deterioration of lipids contained in the salmon heads during frozen storage. Similar to the previous study at 10˚C, increased amounts of free fatty acid in the oil extracted from ground heads stored at -18˚C suggested that the increased availability of endogenous enzymes in contact with lipids occurring during the grinding process promoted the hydrolysis of lipids. The rate of formation of free fatty acids in the oil extracted from heads stored at -18˚C was slower than the rate of free fatty acids formed at 10˚C storage in the oil extracted from salmon heads, which confirmed the hydrolytic activity of lipases under freezing conditions although at a slower rate than under chilling conditions. In this experiment, the added antioxidant did not affect the lipid hydrolysis of salmon heads.

Wu et al. (2020) reported lowering the lipid oxidation in herring (*Clupea harengus*) by-products with added antioxidants by rinsing and mincing methods, indicating the effectiveness of adding antioxidants directly on the surface of the fish by-products on the oxidation of lipids during ice-storage. The oil quality was maintained for 12 days (no peroxides or TBARS were formed) in the samples with the added antioxidant by the rinsing method. The samples without added antioxidant

showed the formation of oxidation products from day 1. In contrast, no oxidation products were formed during storage in the samples with the added antioxidant by the mincing method, indicating an efficient addition of the antioxidants by rinsing and mincing methods. In the present study, adding the antioxidant by the grinding method led to lower oxidative stability of the lipids present in the salmon heads than the oxidative stability observed with the addition of antioxidants by the tumbling method. A possible explanation could be the additional step of mincing applied to the by-products after rinsing, which could have increased the oxygen level in the samples, thus increasing their susceptibility to oxidation during storage. Furthermore, in the present study, the gills were excluded from salmon heads, which could have significantly reduced the hemoglobin in the system and thereby reduced the hemoglobin-mediated lipid oxidation. During the same study, Wu et al. (2020) analyzed the hemoglobin content in herring (*Clupea harengus*) heads, backbones, caudal fin, and residuals (including intestines, skin, and eggs) and reported a significant amount of hemoglobin in herring heads (70.9 μM/kg sample) compared to the backbone, caudal fin, and residuals (42.8, 39.1, and 40.6 μM/kg sample, respectively), which the authors attributed to a higher number of blood vessels present in the gills for oxygen transport to the fish body. In the present study, the potentially reduced amount of hemoglobin in salmon heads provided by the absence of gills (reactive organs) could have prevented the formation of a higher level of lipid oxidation during storage.

4.3.2. Effect of antioxidant addition on salmon frames

The quality of the oil extracted from salmon frames, with and without the addition of antioxidants, was analyzed on days 1, 30, 60, and 90 of storage (Table 12). The Tukey's pairwise comparisons of the oil quality between treatments and time are presented in Figures 37 to 40.

Table 12. Quality analysis of oil extracted from salmon frames, with and without antioxidant (AOX), stored at -18˚C for up to 90 days. The means showing different letters are statistically different ($p<0.05$).

Treatment	Peroxide value (meq/kg oil) (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled frames	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^{cdef}	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 (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled frames	0.70 ± 0.05^{bcd}	0.19 ± 0.15^{cde}	1.69 ± 0.13^{a}	0.28 ± 0.23^{bcde}
Tumbled frames + AOX	0.74 ± 0.2^{bc}	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 (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled frames	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 (%) (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled frames	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}

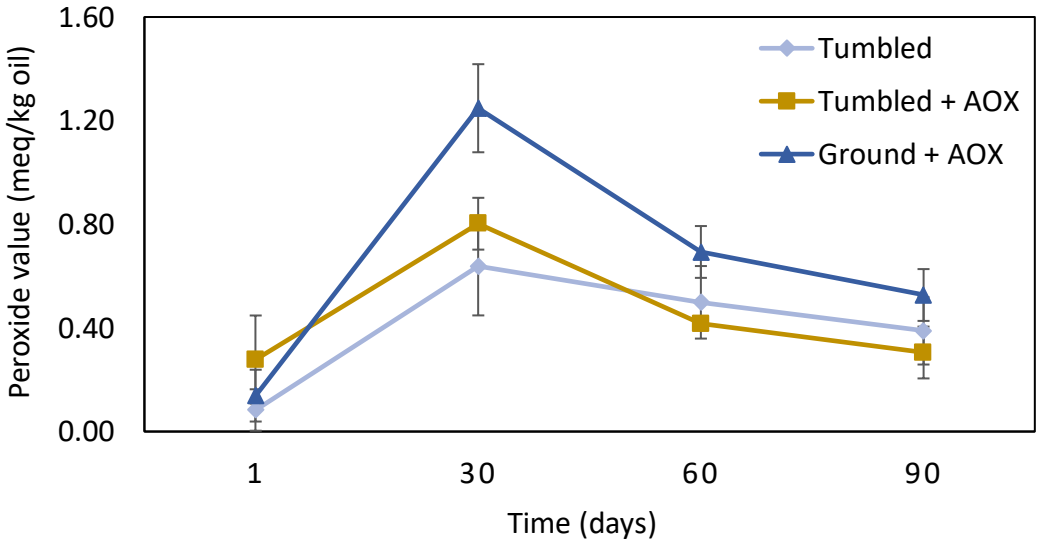


Figure 37. Peroxide values of oil extracted from salmon frames stored at -18˚C for up to 90 days. Values represent mean ±SEM.

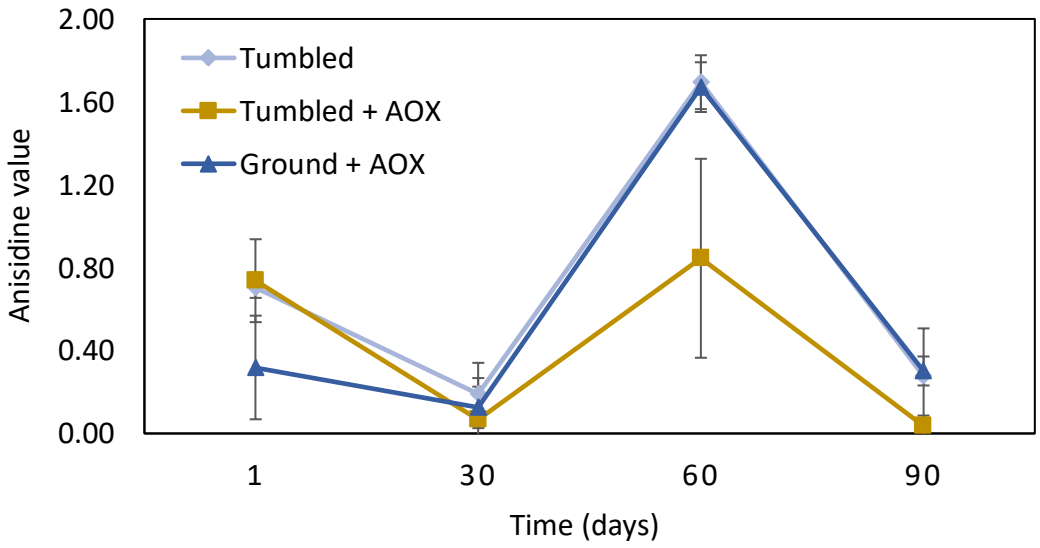


Figure 38. *p*-Anisidine values of oil extracted from salmon frames stored at -18˚C up to 90 days. Values represent mean ±SEM.

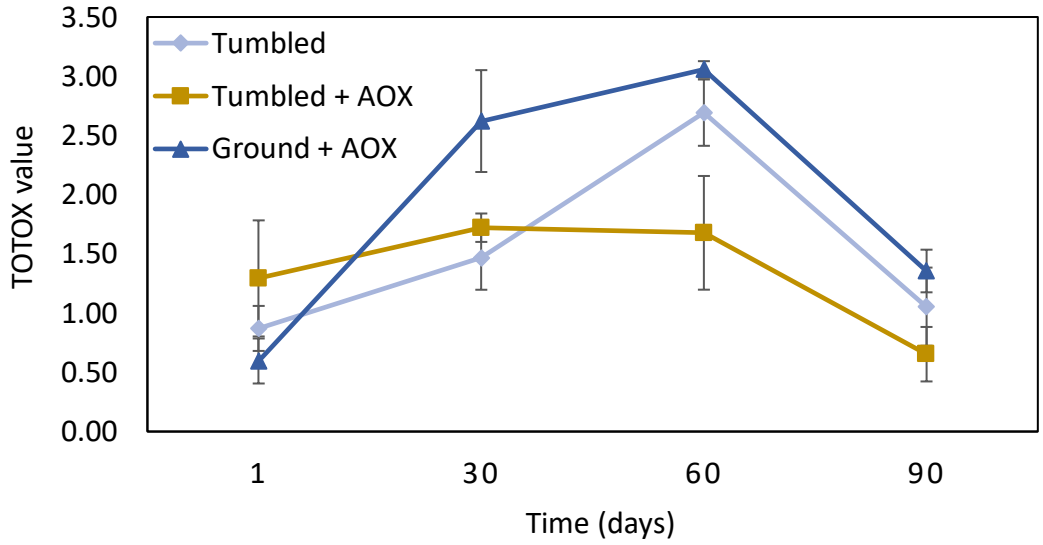


Figure 39. TOTOX values of oil extracted from salmon frames stored at -18˚C up to 90 days. Values represent mean ±SEM.

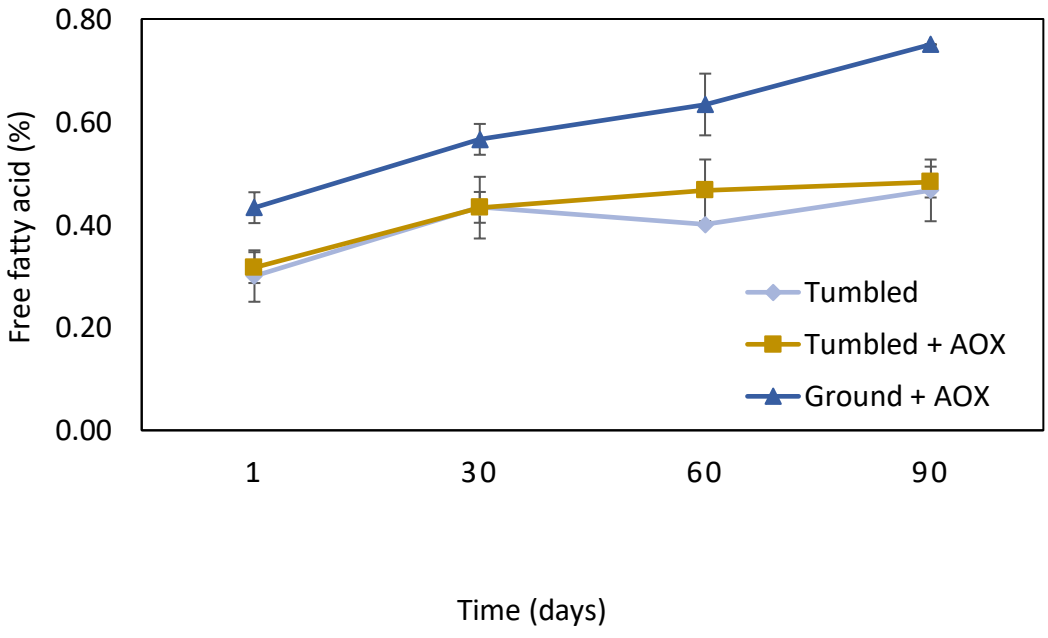


Figure 40. Free fatty acid % of oil extracted from salmon frames stored at -18˚C up to 90 days. Values represent mean ±SEM.

The effect of the antioxidant addition on the oxidation of oils extracted from tumbled and ground frames was similar to the effect recorded on the oxidation of salmon heads during the frozen study. The addition of an antioxidant to tumbled frames improved lipid quality. The grinding process promoted the lipid oxidation of salmon frames, even with the added antioxidant. The peroxide values in the oil extracted from tumbled frames with and without the addition of the antioxidant were below 1 meq/kg oil. In comparison, the peroxide value recorded a peak of 1.25 meq/kg in the oil extracted from ground frames with the added antioxidant on day 30, which was significantly higher at the 0.05 significance level compared to the oil extracted from the tumbled frames.

After day 30, the amount of peroxides in the three treatments decreased, suggesting the progress of lipid oxidation. The range of peroxides observed in the study of the frames was similar to the range of peroxides reported in salmon heads.

The *p*-Anisidine value obtained in the oil extracted from tumbled frames without the addition of the antioxidant and oil extracted from the ground frames with the antioxidant addition exhibited a peak with a similar value on day 60 (1.69 and 1.67, respectively). In contrast, the *p*-Anisidine value (0.85) of the oil extracted from the tumbled frames with added antioxidant was significantly lower at the 0.05 significance level on the same day, suggesting a protective effect of both the antioxidant (compared to tumbled frames without added antioxidant) and the tumbling method (compared to ground frames with added antioxidant) on the oxidation of lipids in the frames. The further decrease of the *p*-Anisidine value in the three treatments on day 90 suggested the lipid oxidation likely progressed.

The total oxidation value of the oil extracted from ground frames with the added antioxidant was significantly higher at the 0.05 significance level than those from tumbled frames on Day 30. The total oxidation value of the oil extracted from tumbled frames with the added antioxidant showed no peaks and was significantly lower at the 0.05 significance level on day 60. Overall, the range total oxidation values developed during storage in all the treatments was below 3.1, which is far less than the quality limit of TOTOX value of 26 permitted for human consumption (Codex Alimentarius Commission, 2017).

The free fatty acid content increase in the oil extracted from the frames followed the same pattern observed in the oil extracted from salmon heads with similar values. The free fatty acid content in oil extracted from the tumbled frames with and without the antioxidant addition slowly increased during storage to a maximum of approximately 0.5% by day 90. The content of free fatty acids in the oil extracted from ground frames added with the added antioxidant was significantly higher at the 0.05 significance level on days 30, 60, and 90, with a maximum of 0.75% on day 90. The release of free fatty acids showed an increasing trend during salmon heads and frames storage. The highest content of free fatty acids in the oil extracted from the frames was 0.75%, similar to the highest in oil extracted from the heads (0.78%), indicating a similar enzyme activity hydrolyzing lipids from salmon heads and frames during frozen storage.

Similar to the findings in salmon heads, the addition of the antioxidant did not affect the lipid hydrolysis of the extracted oil from the salmon frames during storage. This finding agrees with other studies indicating a lack of association between antioxidants and lipid hydrolysis and a lack of correlation between lipid oxidation and lipolysis when inhibiting oxidation with antioxidants (Albertos et al., 2015; Sabeena Farvin et al., 2012).

Wu et al. (2021) studied the lipid oxidation in mechanically separated muscle (MSM) extracted from herring backbones with the addition of an antioxidant (rosemary extract-based) at 0.5% and reported an inhibited lipid oxidation during 11 days of ice storage. In the same study, MSM from Atlantic salmon backbones with and without the addition of antioxidant showed oxidative stability (no significant peroxide value and TBARS observed) during 11 days of ice storage, while MSM extracted from Atlantic salmon backbones without antioxidant were stable during storage at -20˚C for 8 months. Similarly, in the present study, regardless of the addition of antioxidant, salmon frames (tumbled, tumbled with antioxidants, ground with antioxidants) showed slight oxidation during 3 months of storage at -18˚C, which was below the quality permissible limits for human consumption (Codex Alimentarius Commission, 2017).

4.3.3. Effect of antioxidant addition in salmon viscera

The quality of the oil extracted from salmon viscera, with and without the addition of antioxidants, was analyzed on days 1, 30, 60, and 90 of storage (Table 13). The Tukey's pairwise comparisons of the oil quality between treatments and time are presented in Figures 41 to 44.

In contrast to salmon heads and frames, salmon viscera exhibited greater lipid oxidation during frozen storage, similar to the cold storage study. The quality deterioration of viscera lipids was prevented using the tumbling method and promoted using the grinding method.

The peroxide values observed in the oil extracted from tumbled viscera with and without added antioxidants was below 0.6 meq/kg oil. The maximum peroxide value observed in the oil extracted from ground viscera with the added antioxidant (1.86 meq/kg oil) was above 3 times the maximum peroxide value in tumbled viscera oil with the added antioxidant (0.58 meq/kg oil), indicating the promotion of lipid oxidation by the grinding method.

Table 13. Quality analysis of oil extracted from salmon viscera, with and without antioxidant (AOX), stored at -18˚C for up to 90 days. The means showing different letters are statistically different ($p<0.05$).

Treatment	Peroxide value (meq/kg oil) (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled viscera	0.08 ± 0.0^{ef}	0.42 ± 0.0^{cde}	0.42 ± 0.0^{cde}	0.36 ± 0.1^{cdef}
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 (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled viscera	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 (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled viscera	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 (%) (±SEM)			
	Day 1	Day 30	Day 60	Day 90
Tumbled viscera	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}

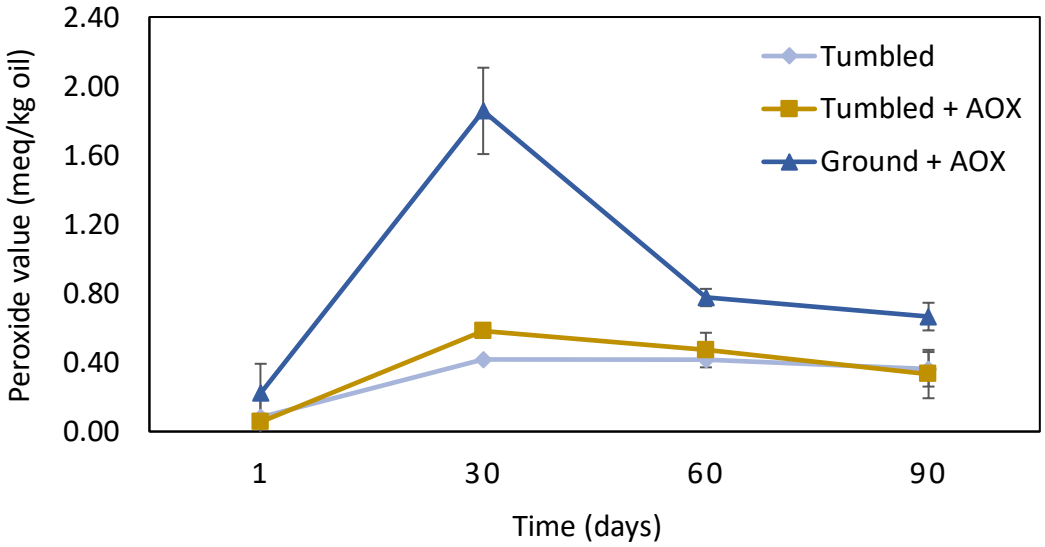


Figure 41. Peroxide values of oil extracted from salmon viscera stored at -18˚C for up to 90 days. Values represent mean ±SEM.

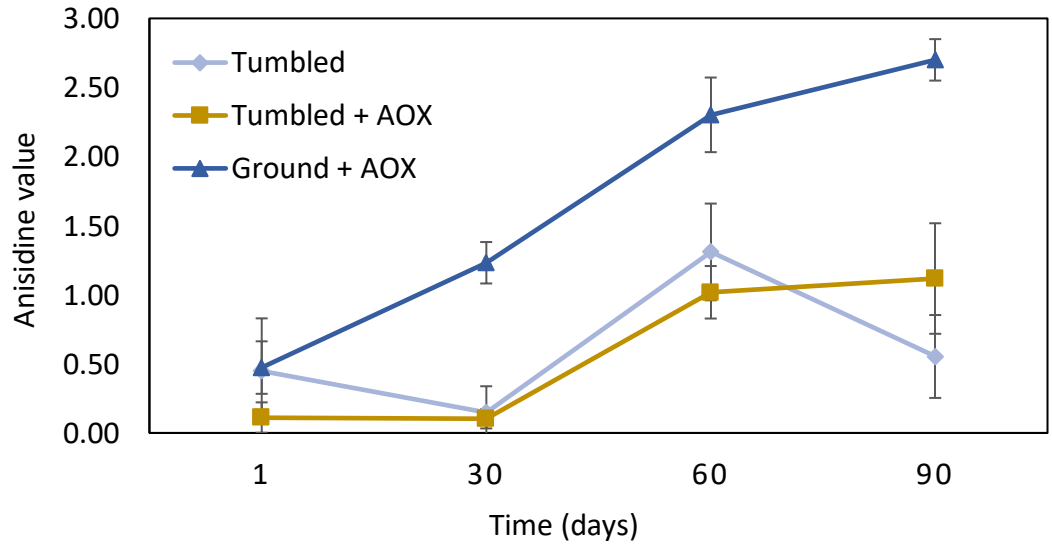


Figure 42. *p*-Anisidine values of oil extracted from salmon viscera stored at -18˚C for up to 90 days. Values represent mean ±SEM.

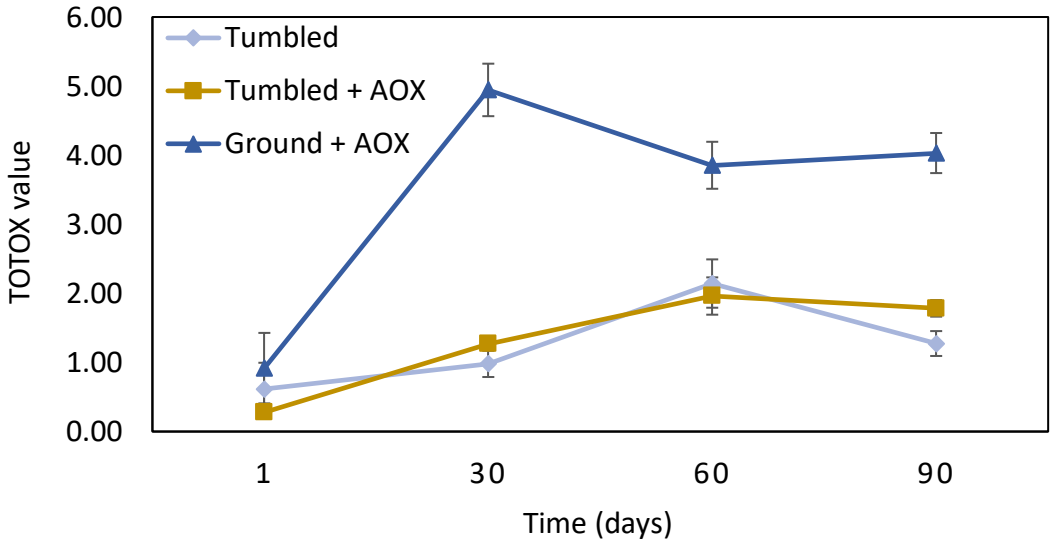


Figure 43. TOTOX values of oil extracted from salmon viscera stored at -18˚C for up to 90 days. Values represent mean ±SEM.

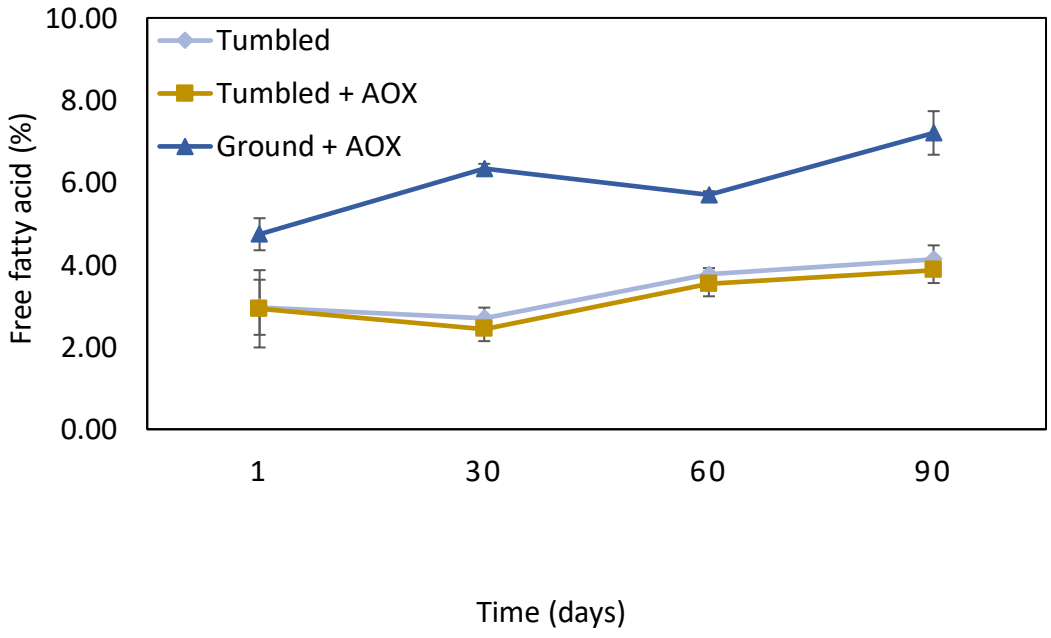


Figure 44. Free fatty acid % of oil extracted from salmon viscera stored at -18˚C for up to 90 days. Values represent mean ±SEM.

After day 30, the amount of peroxides in the ground viscera oil with the added antioxidant decreased to 0.78 and 0.67 meq/kg on days 60 and 90, respectively, suggesting the progress of the lipid oxidation. The tumbling method promoted the generation of the same range of peroxide values in the viscera oils as those obtained in the oils extracted from heads and frames during storage (including all treatments, tumbled, ground, with and without added antioxidant). In contrast, the grinding method increased susceptibility to oxidation of the viscera lipids during storage.

The analysis of *p*- Anisidine values revealed the impact of the grinding process on the second stage of lipid oxidation. The *p*-Anisidine values of the oils extracted from tumbled viscera with and without added antioxidant increased to 1.31 and 1.02, respectively, on day 60 and then decreased by day 90, following the same trend observed previously in the *p*-Anisidine values of oils extracted from heads and frames. Contrarily, the *p*-Anisidine value recorded in the oil from ground viscera constantly increased during the entire study, with significantly higher values at the 0.05 significance level on days 30, 60, and 90, indicating more extensive oxidation than that observed in the tumbled viscera oils.

The TOTOX value obtained in the oil extracted from ground viscera with the added antioxidant was significantly higher than the TOTOX values in oils extracted from tumbled viscera with and without the addition of the antioxidant. The addition of the antioxidant did not reduce the total oxidation developed in the oil extracted from tumbled viscera. However, the grinding method significantly increased the total oxidation at the 0.05 significance level of viscera oi with the added antioxidant on days 30, 60, and 90. A maximum TOTOX value of 4.94 was observed in ground viscera oil on day 30. The value was below the quality limit of 26, established for human consumption (Codex Alimentarius Commission, 2017).

A high free fatty acid content is usually the limiting factor for using salmon viscera in producing value-added products such as nutraceutical grade or food-grade oil and biodiesel, even when other lipid parameters such as peroxide value are below the quality limits of food-grade oil (Liu et al., 2021; Mohiddin et al., 2021). The stabilization of the viscera lipids during storage is challenging since various digestive enzymes in the viscera promote the hydrolysis of the lipids, which take part in the lipid deterioration process (Kurtovic et al., 2009; Sae-Leaw & Benjakul, 2017; Smichi et al., 2013). Heating has been widely used as an effective method to inactivate enzymes. However, the heating process also promotes the deterioration of other potential bio-compounds in fish materials such as proteins and lipids (Dave et al., 2014). During the present study, the free fatty acid content in the oil extracted from tumbled viscera with and without the added antioxidant ranged from 2.43 to 4.13% on day 90, while the free fatty acid content in the ground viscera oil with the added antioxidant increased from 4.74% on day 1 to 7.2% on day 90, exceeding the maximum acceptable limit of 7% (Bimbo, 1998). The oil extracted from ground viscera with the added antioxidant significantly increased at the 0.05 significance level in the free fatty acid contents on days 1, 30, 60, and 90, compared to the oil extracted from tumbled viscera with and without added antioxidant, indicating the promoting effect of the grinding method on the lipid hydrolysis of salmon viscera. A prolonged deterioration of the viscera lipids could be expected over increased storage time.

In this study, the added antioxidant did not affect the oxidation or hydrolysis of the viscera lipids. Liu et al. (2021) evaluated the effect of an antioxidant (rosemary-based) added to salmon (*Salmo salar*) viscera and a mix of heads and frames before oil extraction and reported a preventive effect of the antioxidant on the lipid oxidation while no effect on the lipid hydrolysis of the extracted oil.

Ozen et al. (2011) 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 and

reported statistically lower oxidation in the mince with the added antioxidant compared with the control (no antioxidant addition), according to the peroxide (2.5 and 5 meq/kg oil) and TBARS (50 and 130 mg MDA/kg sample) values. The antioxidant effect of the grape seed extract was related to the total phenolic content (66 g gallic acid/kg extract) and antioxidant activity (156 mM Trolox/g extract). The antioxidant showed a preventive effect on the lipid oxidation of mackerel mince during storage. Contrastingly, in the present study, the antioxidant showed a slight preventive effect on the lipid oxidation of salmon by-products during storage, possibly due to more extensive lipid oxidation developed in the mackerel mince than the oxidation developed in the salmon by-products in the present study.

Cropotova et al. (2020) 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 oil and TBARS of 5.4 mM/100g after storage, indicating the progression of lipid oxidation in the mince, which contained 12.2-13.4% of lipids (wet basis). The lipid oxidation reported in the Atlantic mackerel was higher than the lipid oxidation reported in the present study, possibly due to the lower oxidative stability of the Atlantic mackerel and oily nature compared to that of the Atlantic salmon by-products used in the present study. The oxidative stability of fish materials is determined by different aspects such as fat level, unsaturation level of the oils, antioxidants, prooxidants, and diet. High fat and prooxidants in the fish and high-marine-oil diets are related to lower oxidative stability in the fish materials. Contrastingly, low fat, high antioxidants, low-marine-oil diets are related to high oxidative stability in the fish materials (Dave & Manuel, 2014; Honold et al., 2016; Wu et al., 2022).

4.3.4. PUFA analysis

The fatty acid profile analysis of the oil extracted from single samples of salmon heads, frames, and viscera, with and without the addition of the antioxidant, was performed by the Aquatic Research Center (ARC), Memorial University of Newfoundland. The content of PUFAs in the oil extracted from single samples of salmon by-products with different treatments is presented in Tables 14 to 16 and Figures 45 to 47, respectively.

As indicated in Table 14 and Figures 45 to 47, the PUFA content remained similar during the storage of salmon heads, frames, and viscera in all the treatments. Salmon heads showed the highest amounts of PUFAs (29.05-30.24), followed by frames (27.66-29.65), and viscera contained the lowest amounts (27.52-28.40). The PUFA content in heads, frames, and viscera remained similar during the experiment regardless of the addition of the antioxidant (with or without antioxidant) and the addition method (tumbling or grinding). No degradation of the PUFAs was associated with lipid oxidation during storage, possibly due to the low lipid oxidation developed during storage and the presence of endogenous and added antioxidants in the salmon by-products. Even the highest deterioration observed in the oil extracted from ground viscera with the added antioxidant showed no degradation of the PUFAs, possibly due to the endogenous and added antioxidants protecting their degradation. Similar to the findings in the present study, Standal et al. (2018) reported no lipid oxidation development and no degradation of long-chain n-3 PUFA in Atlantic mackerel (*Scomber scombrus*) stored for 12 months at -27˚C. Wu and Bechtel (2008) reported the development of free fatty acids in Alaska pink salmon (*Oncorhynchus gorbuscha*) heads and viscera stored for 4 days at 6 and 15˚C, while PUFA, EPA, and DHA remained similar during the storage period. Later, Wu and Bechtel (2009) reported the development of free fatty acids during the storage of walleye pollock (*Theragra chalcogramma*)

Table 14. Total PUFA content of oil extracted from salmon heads, frames, and viscera, with and without antioxidant (AOX), stored at -18˚C for up to 90 days.

Treatment	PUFA (g/100g oil) *			
	Day 1	Day 30	Day 60	Day 90
Tumbled heads	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	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	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

* Analysis from single samples.

Table 15. Primary PUFAs contained in oil extracted from salmon heads, frames, and viscera with and without antioxidant (AOX) stored at -18˚C, obtained at day 1 of storage.

Treatment	Fatty Acid (g/100g oil) *				
	Linoleic acid	ALA	DHA	EPA	DPA
Tumbled heads	14.52	4.16	2.86	2.76	1.11
Tumbled heads +AOX	14.35	4.05	2.60	2.55	0.99
Ground heads +AOX	14.38	4.02	2.98	2.72	1.07
Tumbled frames	14.89	3.99	2.01	2.22	0.88
Tumbled frames +AOX	14.75	4.01	2.11	2.19	0.95
Ground frames +AOX	14.22	3.51	2.79	1.88	1.04
Tumbled viscera	14.76	3.99	1.76	1.87	0.92
Tumbled viscera +AOX	15.41	4.32	1.62	1.96	0.91
Ground viscera +AOX	15.19	4.18	1.79	1.87	0.91

* Analysis from single samples.

Table 16. Total PUFA content and primary fatty acids contained in oil extracted from salmon heads, frames, and viscera, with and without antioxidant (AOX), stored at -18˚C for up to 90 days.

PUFAs (g/100g oil) *	Treatment											
	Tumbled				Tumbled + AOX				Ground + AOX			
	D 1	D 30	D 60	D 90	D 1	D 30	D 60	D 90	D1	D 30	D 60	D 90
Heads												
∑ PUFAs	29.77	29.38	29.56	29.31	29.05	30.24	29.36	29.16	30.03	29.52	29.31	29.58
Linoleic acid	14.52	14.31	14.48	14.42	14.35	14.70	14.42	14.81	14.38	14.46	14.41	14.46
ALA	4.16	3.85	4.00	4.05	4.05	4.29	4.06	4.26	4.02	4.09	4.04	4.08
DHA	2.86	2.82	2.69	2.82	2.60	2.71	2.42	2.29	2.98	2.68	2.66	2.70
EPA	2.76	2.60	2.78	2.51	2.55	2.92	2.73	2.60	2.72	2.61	2.57	2.62
DPA	1.11	1.05	1.12	1.04	0.99	1.04	1.02	0.90	1.07	1.01	1.01	1.02
Frames												
∑ PUFAs	28.81	29.04	28.08	28.37	28.36	27.66	28.32	28.63	28.00	28.39	28.65	29.55
Linoleic acid	14.89	15.03	15.28	15.38	14.75	14.79	15.03	15.05	14.22	14.77	14.91	15.10
ALA	3.99	4.18	3.98	4.24	4.01	3.84	3.97	4.35	3.51	3.98	4.05	4.17
DHA	2.01	2.22	1.96	1.60	2.11	1.91	2.03	1.73	2.79	2.09	2.09	2.29
EPA	2.22	2.22	1.92	2.11	2.19	2.04	1.90	2.24	1.88	2.18	2.24	2.38
DPA	0.88	0.97	0.93	0.87	0.95	0.86	1.10	0.88	1.04	0.96	0.98	1.00
Viscera												
∑ PUFAs	27.52	28.40	28.03	28.05	28.23	28.34	28.37	28.17	28.06	27.76	28.16	28.32
Linoleic acid	14.76	15.33	15.05	15.32	15.41	15.42	15.30	15.51	15.19	15.04	15.48	15.34
ALA	3.99	4.20	4.16	4.17	4.32	4.33	4.27	4.31	4.18	4.18	4.35	4.32
DHA	1.76	1.86	1.81	1.72	1.62	1.62	1.70	1.55	1.79	1.75	1.73	1.76
EPA	1.87	1.86	1.86	1.90	1.96	1.85	1.89	1.86	1.87	1.90	1.91	1.92
DPA	0.92	0.84	0.96	0.83	0.91	0.89	1.03	0.92	0.91	0.92	0.90	0.91

* Analysis from single samples.

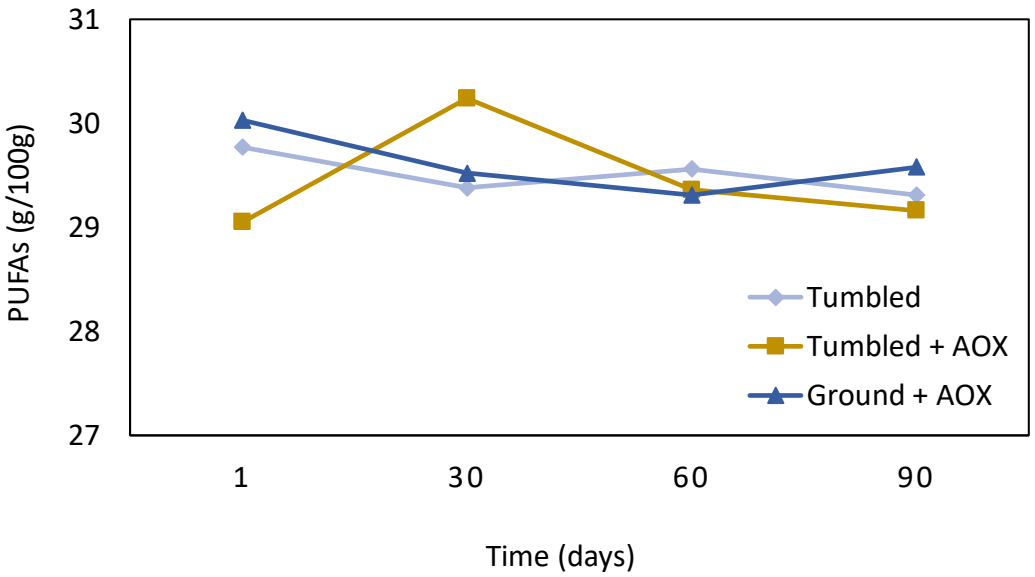


Figure 45. PUFA content of oil extracted from salmon heads stored at -18˚C for up to 90 days. Values represent single samples.

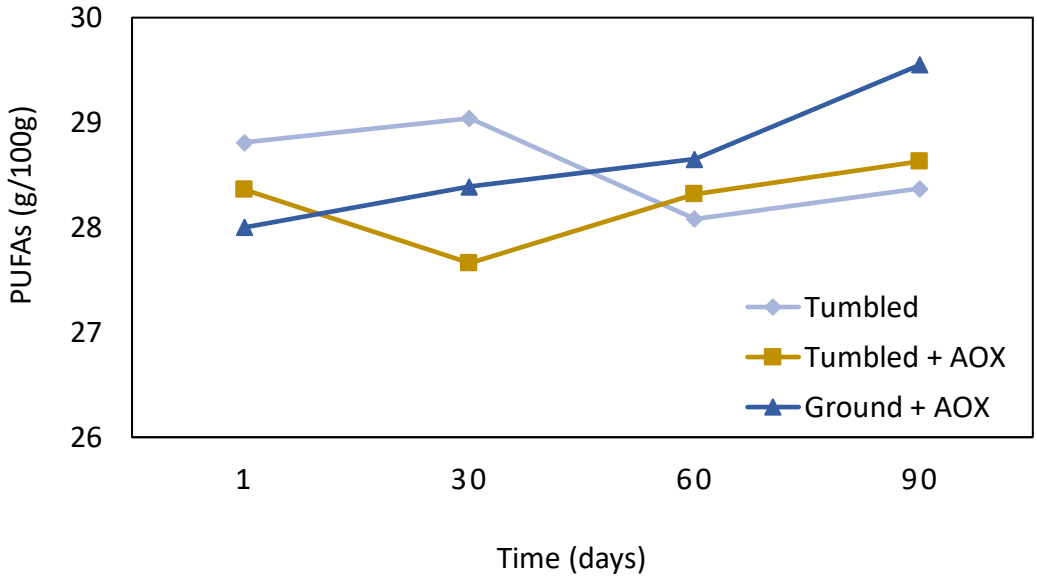


Figure 46. PUFA content of oil extracted from salmon frames stored at -18˚C for up to 90 days. Values represent single samples.

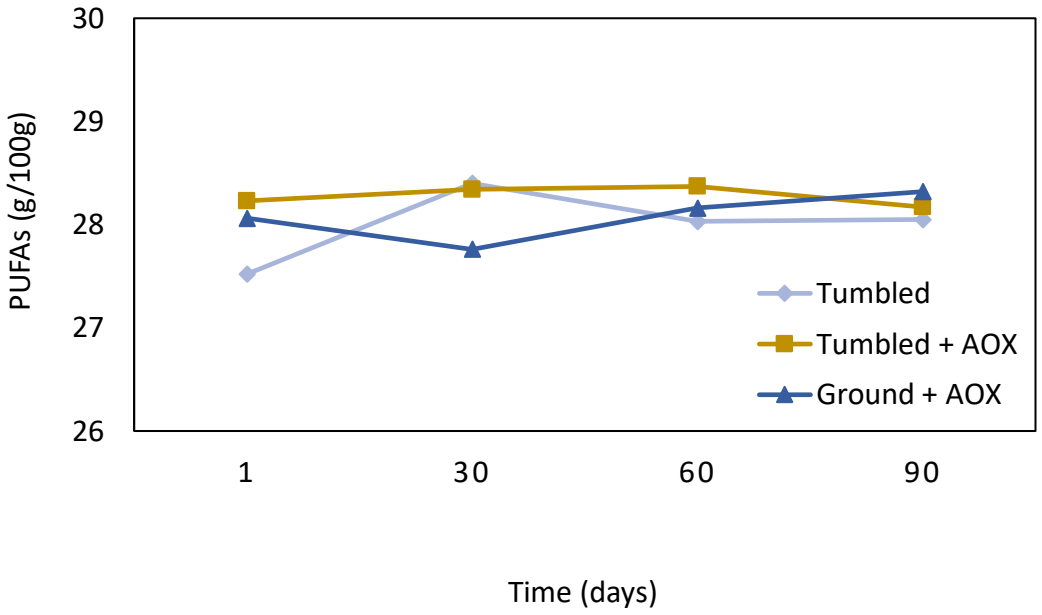


Figure 47. PUFA content of oil extracted from salmon viscera stored at -18˚C for up to 90 days. Values represent single samples.

by-products (heads, frames, skin, and viscera), while PUFA, EPA, and DHA remained similar during 4 days of storage at 15˚C and 10 days of storage at 6˚C.

As indicated in Table 15, the primary PUFAs present in the oil extracted from salmon heads, frames, and viscera, with contents above 1 g/kg oil, were: linoleic acid (18:2ω6), alpha-linolenic acid (ALA, 18:3ω3), eicosapentaenoic acid (EPA, 20:5ω3), docosahexaenoic acid (DHA22:6ω3), and docosapentaenoic acid (DPA, 22:5ω3). The predominant fatty acid was linoleic acid, constituting nearly half of the total PUFA content in all the by-products (ranging 14.22 to 15.41 g/100g oil), followed by alpha-linolenic acid (ranging from 3.51 to 4.32 g/100g oil), docosahexaenoic acid (from 1.62 to 2.98 g/100g oil), eicosapentaenoic acid (from 1.87 to 2.76 g/100g oil), and docosapentaenoic acid (from 0.88 to 1.11 g/100g oil).

While the content of linoleic acid, ALA, and DPA was more constant among by-products, the amounts of EPA and DHA were slightly different. Salmon heads contained the highest amounts of EPA and DHA (5.14-5.62 g/100g oil), followed by frames (4.23-4.67 g/100g oil) and viscera (3.58-3.65 g/100g oil). The difference in PUFA content in heads, frames, and viscera are likely related to the amount of EPA and DHA present in each by-product, which was similar between treatments (tumbled, ground, with and without the addition of antioxidant) of the same by-product. As indicated in Table 16, linoleic acid, ALA, and DPA remained similar during storage. However, more variation was observed in the contents of EPA and DHA, as slight decreases in DHA were observed primarily in the oil extracted from frames (all treatments) and followed by n the oil extracted from tumbled and ground heads with the added antioxidant. EPA slightly decreased in the oil extracted from the tumbled heads without adding the antioxidant and increased in the oil extracted from the ground frames with added antioxidant.

Furthermore, the highly unsaturated fatty acids, EPA and DHA, present in the viscera oils, were more stable during storage. This could be due to the antioxidants contained in the fish feed remaining in the digestive tract of the viscera. As the total PUFAs remained constant during storage, the changes observed in EPA and DHA are likely attributed to causes such as enzymatic and microbial activity rather than entirely to oxidation reactions.

While some authors reported high PUFAs in fatty fish, others found lower levels even within the same species. Dave et al. (2014) analyzed the fatty acid profile of oil extracted from farmed Atlantic salmon heads, frames, and viscera and reported approximately 39% PUFA in the oil, an average of 8.21-8.49% DHA and 8.66% EPA in salmon heads and frames, and 7.53% and 7.41% of EPA and DHA in salmon viscera, respectively. In contrast to the present study, their results suggested that the contents of PUFA, DHA, and EPA were higher, and the PUFA contents were constant among by-products. However, a higher content of EPA and DHA in heads and frames compared to viscera is consistent with the findings in the present study. In another study, Dave and Manuel (2014) reported 32.23% of PUFA, 3.48% of DHA, and 4.63% of EPA in farmed Atlantic salmon (*Salmo salar*) viscera oil, which are also above the contents found in the oil extracted from viscera in the present study. Dave (2016) characterized the oil from farmed Atlantic salmon (*Salmo salar*) frames and reported 27.48% PUFA, 2.02% DHA, and 0.75% EPA, suggesting the low amount of EPA and DHA possibly originated from a commercial diet low in marine oil. Routray et al. (2017) analyzed the oil from farmed Atlantic salmon (*Salmo salar*) 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 and attributing the difference to reasons such as commercial diets. Later, Dave and Routray (2018) studied the oil quality from farmed Atlantic salmon (*Salmo salar*) frames and reported 29.05% PUFA, 2.13% DHA, and 1.94% EPA.

In studies performed on other fatty fish species, Aidos et al. (2003) reported an average of 18.6%, 6.7%, and 5.3% of PUFA, DHA, and EPA in wild herring by-products. Wu and Bechtel (2008) reported an average of 34.5%, 12.9%, and 10.6% of PUFA, DHA, and EPA in wild Alaska pink salmon (*Oncorhynchus gorbuscha*) heads and viscera. Honold et al. (2015) studied the oil quality from farmed rainbow trout (*Onchorhynchus mykiss*) fed diets high in vegetable and marine oil and reported higher levels of EPA and DHA in high marine oil-fed fish (8.69% and 3.7%) compared to high vegetable oil-fed fish (3.37% and 1.21%). The amount of EPA and DHA found in fish is correlated to the amount of these fatty acids contained in the diet provided to the fish. Due to a variety of factors such as economic cost and source availability, marine oils have been replaced by vegetable oils in commercial fish diets, modifying the lipid profile and the content of EPA and DHA in the fish (Moxness Reksten et al., 2022; Regost et al., 2004; Sprague et al., 2020).

CHAPTER 5. CONCLUSIONS

The effect of the presence of reactive organs and processing methods (high and low processing) on the lipid oxidation of the oil extracted from salmon by-products during storage at 10˚C for up to seven days was studied. The effect of antioxidant addition on the lipid oxidation of the oil extracted from salmon by-products during storage at -18˚C for up to 90 days was studied. The PUFA content was investigated in the by-products from the study at -18˚C for up to 90 days. Farmed Atlantic salmon heads, frames, and viscera were studied separately.

Overall, the presence of reactive organs promoted lipid deterioration in the oil extracted from salmon heads and viscera during storage at 10˚C for up to seven days. The high processing (grinding process) promoted lipid deterioration in the oil extracted from salmon heads, frames, and viscera during storage at 10˚C for up to seven days and at -18˚C for up to 90 days. The addition of an antioxidant to tumbled and ground heads, frames, and viscera to which reactive organs were excluded seemed unnecessary during storage at -18˚C for up to 90 days, as the lipid oxidation observed in the oil extracted from the samples with and without added antioxidant was low, possibly due to the low contents of highly unsaturated fatty acids present in the oils.

The presence of reactive organs and the grinding process significantly affected the peroxide value, the *p*-Anisidine value, and the TOTOX value of the oil extracted from Atlantic salmon by-products stored at 10˚C for up to seven days at the 0.05 significance level. Viscera appeared to be the most affected of the three by-products. The oil extracted from ground viscera almost reached the permissible quality limits for peroxides (Codex Alimentarius Commission, 2017). A 200% increase of TOTOX value and a 50% increase of FFA were observed in the oil extracted from viscera with reactive organs, as opposed to the oil extracted from viscera without reactive organs.

Further, a 40% increase of *p*-Anisidine value, 100% increase of TOTOX value, and 200% increase of FFA were observed in the oil extracted from ground viscera, as opposed to the oil extracted from tumbled viscera.

The antioxidant added by the low processing (tumbling) method had no significant effects on the peroxide value or the free fatty acid content of the oil extracted from tumbled heads, frames, and viscera stored at -18˚C for 90 days (at the 0.05 significance level). However, the *p*-Anisidine value and the TOTOX value of the oil extracted from tumbled heads and frames with added antioxidant was reduced (at the 0.05 significance level), as opposed to the oil extracted from tumbled heads and frames without added antioxidant.

The antioxidant added by the high processing (grinding) method promoted a significant increase of the peroxide value, TOTOX value, and FFA content of the oil extracted from heads, frames, and viscera, and the *p*-Anisidine value of the oil extracted from frames and viscera (at the 0.05 significance level), as opposed to the oil extracted from heads, frames, and viscera added with antioxidant by the low processing (tumbling) method. The free fatty acid levels in the ground viscera exceeded the permissible quality limits (Bimbo, 1998).

The content of PUFAs remained similar in the oil extracted from tumbled and ground heads, frames, and viscera with and without antioxidant stored at -18˚C for 90 days. Salmon heads averaged the highest PUFA content (29-30g/100g oil), frames averaged 28-29g PUFA/100g oil, and viscera averaged the lowest PUFA content (27.7-28.3g/100 g oil). The content of DHA plus EPA averaged 4g/100 g in oil extracted from tumbled and ground heads, frames, and viscera with and without antioxidant, considered low compared to previous studies.

The pathway for the preservation of salmon by-products provided in this research represents an opportunity for the aquaculture sector to maximize the value of this underutilized resources through the extraction of valuable compounds such as fish oil for the nutraceutical and pharmaceutical industry.

5.1. Future work and recommendations

The presence of reactive organs affected the lipid oxidation and lipolysis of salmon by-products during storage at 10˚C up to seven days. This study should be extended to frozen salmon heads and viscera to evaluate the impact of sorting and excluding reactive organs on the lipid quality and oxidative stability of salmon by-products during frozen storage. Further, the rate of oxidation should be studied more closely, including additional analysis points throughout the storage time periods.

The grinding process promoted lipid oxidation and lipolysis on the oil extracted from salmon heads, frames, and viscera stored at 10˚C for up to seven days and -18˚C for up to 90 days. The grinding process should be avoided before storage when lipid degradation is undesirable.

All the treatments including tumbling without reactive organs-without antioxidant, tumbling without reactive organs-with antioxidant, and grinding without reactive organs-with antioxidant) were effective for the storage of salmon by-products (salmon heads, frames, and viscera) at 18˚C for up to 90 days. However, a longer storage should be conducted to investigate the maximum storage time of salmon by-products in which the original quality (e.g., level of EPA and DHA) could be maintained.

According to previous studies (Dave et al., 2014; Dave & Manuel, 2014; Wu & Bechtel, 2008), the overall levels of EPA and DHA extracted from the salmon by-products in this study were low. The effect of the presence of reactive organs, processing methods, and added antioxidants on the lipid oxidation of by-products should be evaluated in by-products with higher content of EPA, DHA, and other highly unsaturated fatty acids (e.g., ALA, DPA) to verify the effectiveness of these methods to increase the oxidative stability of by-products in samples which are more susceptible to lipid oxidation. Lipid class-analysis (TAG, sterols, phospholipids, etc.) performed on salmon by-products could be useful in the characterization of by-products lipid oxidation. This analysis could provide information related to lipids which are more susceptible to oxidation, with the possibility to broadly apply these findings on other similar waste resources.

The need for an added antioxidant was not apparent in the frozen storage study, possibly due to a high content of endogenous antioxidants present in the salmon by-products. The antioxidant strength in the by-products provided by the added and the endogenous antioxidants should be investigated through an antioxidant activity test using methods such as photochemiluminescence (Wu & Bechtel, 2008), DPPH radical scavenging activity, hydroxyl radical scavenging activity, and metal chelating ability (Nikoo et al., 2020) to understand the oxidative stability observed during storage and observe the correlations (if any) of the antioxidant activity with lipid oxidation and PUFAs content.

Salmon viscera was the most affected by-product by lipid oxidation and lipolysis. The inactivation of endogenous enzymes should be studied to avoid elevated contents of free fatty acids derived from the enzymatic activity, to enhance lipid quality. Low-heat or no-heat methods such as high hydrostatic pressure should be evaluated for the inactivation of the endogenous enzymes to avoid heat-mediated lipid oxidation being generated during the process.

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