Utilization of by-products from the salmon industry

The chemical composition and storage stability of Atlantic salmon (*Salmo salar*) heads

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2019

Thesis for the degree of Master of Science in Food science

UNIVERSITY OF ICELAND
SCHOOL OF HEALTH SCIENCES
FACULTY OF FOOD SCIENCE AND NUTRITION

Nýting hliðarafurða úr laxavinnslu

Greining á efnasamsetningu og stöðugleika laxahöfða

(*Salmo salar*)

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2019

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60 ECTS

Október 2019

Ritgerð til meistaragráðu í Matvælafræði

HÁSKÓLI ÍSLANDS
MATVÆLA- OG NÆRINGARFRÆÐIDEILD

Prentun: Háskólaprent

Reykjavík, Ísland, 2019

Ágrip

Fyrri rannsóknir hafa gefið til kynna að nauðsynlegt sé að nýta hliðarhráefni úr laxavinnslu betur en nú er gert. Þekking á vinnslumöguleikum þessa hráefnis fer aukandi, en þó endar meirihluti hliðarafurða í vinnslu ódýrra afurða eða er jafnvel fleygt.

Meginmarkmið verkefnisins var að rannsaka hvernig nýta megi hausa úr eldislaxi á Íslandi með því að afla þekkingar á efnasamsetningu og eðliseiginleikum mismunandi hluta hausanna og stöðugleika þeirra í frostgeymslu.

Verkefninu var skipt í þrjá meginþætti. Í fyrsta hluta verkefnisins var nýting og efnasamsetning (vatn, fita, prótein, aska, fríar fitusýrur, fosfólípíð og oxunarafleiður) greindar í heila, gellum, augum, uggum og tálknum laxafiska. Í öðrum parti verkefnisins var stöðugleika þessara innihaldsefna fylgt eftir í gegnum frostgeymslu í fjóra mánuði. Í þriðja og lokahluta verkefnisins var skoðað hvort nýta mætti laxahausana til kollagenútdráttar.

Efnainnihald haushlutanna var æði misjafnt, en það var stöðugt í frostgeyslu við -25°C. Heilinn, gellurnar og augun reyndust vera fituríkir vefir, og gellurnar og augun gætu nýst vel til vinnslu á lípíðum vegna nýtingar og stærðar þessara vefja og því hve einfalt var að fjarlægja þá úr hausunum. Vefir með hærra hlutfall beina, s.s. tálkn og uggar laxanna, innihéldu meira prótein og ösku en mýkri vefir hausanna, s.s. augu eða heili. Tálknin innihéldu einnig áhugaverð steinefni, svo sem sínk og kalk. Uggarnir voru sérstaklega viðkvæmir fyrir fituoxun. Tálknin og augun urðu einnig fyrir oxun við frostgeymsluna. Kollagen sem unnið var úr heilum laxahausum var gulleitt og ásættanlegar heimtur við útdráttinn fengust (6.4%).

Niðurstöður verkefnisins sýndu að nýtingarmöguleikar laxahausa eru mun víðari en áður var haldið og að vinnsla hvers parts haussins (tálkna, auga, heila, gella, ugga) gæti ýtt undir verðmætasköpun í laxavinnslu.

Abstract

Previous studies have indicated the current situation and the need of utilizing by-products from the salmon industry. Awareness of the potential values behind these by-products is rising, but most of the by-products ended up in low-value silage for animal feeding or simply wasted.

The main goal of this project is to investigate the possible utilization of heads from locally farmed Atlantic salmon (*Salmo salar*) in Iceland. Obtaining knowledge of the physiochemical properties of the different parts from salmon heads and their quality and stability as affected by frozen storage conditions are a prerequisite for further value-adding.

There were three major parts of this project. The first part was to determine the yield, content of water, protein, ash, lipid, fatty acid, free fatty acid, phospholipid, peroxide, thiobarbituric acid reactive substances in brain, tongue, eyes, pectoral fins, and gills. The second part was to trace these contents for those five parts individually every two months from October 17th, 2018 to February 26th, 2019. The third part was an attempt to extract collagen from 5 whole salmon heads.

The contents of water, lipid, protein and ash had wide ranges among the five analyzed parts and water, protein and ash were stable for all five parts during the 4-month storage at -25°C. Brain, tongue and eyes showed high amount of lipid, and tongue and eyes could be interesting sources for lipid extraction considering the size and removability. Bony tissues like tongue, fins and gills contained more protein and ash than soft tissues like brain and eyes. And gills possessed large varieties of interesting minerals like zinc and calcium, and the content of calcium was high. Fins were very prone to lipid oxidation and the major part of primary oxidation was finished already before the samples were sent to Matís. Gills and eyes also had high level of both primary and secondary oxidation during the 4-month storage. Collagen extracted from whole salmon head was slightly yellowish and had a relatively satisfying yield of 6.4%.

The results above revealed the potential value behind salmon heads to some extent and processing these parts separately in line with their physiochemical properties would lead to the value creation from salmon head.

Key words: salmon head, by-products, physicochemical properties, potential value.

Acknowledgements

This research was conducted at Matís ohf. In Iceland. I would like to acknowledge Matís ohf. for providing laboratory facilities for the reseach, Eðalfiskur ehf for the raw material. The study was financed by the AVS (Added Value of Seafood) fund from the Ministry of Fisheries and Agriculture in Iceland, the project “Increased value of Icelandic aquaculture” (project number R 17 026-17), which is gratefully acknowledged. I would like to thank my supervisors Sigurjón Arason and María Guðjónsdóttir for their guidance during this study. I would also like to thank Hildur Inga Sveinsdóttir, Cécile Dargentolle and Ulla-Maija Poranen, for their generous help. I also send my gratitude to the staff of the chemical lab at the Matis for the analysis of ash and protein content.

Finally, I want to thank my family for all the support throughout my two-year study abroad.

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Abbreviations

DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FFA	Free fatty acids
FPH	Fish protein hydrolysate
FPI	Fish protein isolate
GC	Gas chromatography
MDA	Malondialdehyde
MUFA	Monounsaturated fatty acids
PL	Phospholipid
PUFA	Polyunsaturated fatty acids
PV	Peroxide value
SFA	Saturated fatty acids
TBARS	Thiobarbituric acid reactive substances

1 Introduction

Salmon is one of the most important commercial species in the world. In 2016, the total production of farmed Atlantic salmon worldwide was around 2 million tons, and it was about 8,000 tons in Iceland (Harvest, 2016). Even though the total catches of salmon are relatively low compared to other species, salmon is very popular because of its high probability of industrialization and nutrition value. However, up to 50% of the total harvest is not used in a proper way due to different postharvest or industrial processes. These parts include the head, skin, blood, backbone, tail, viscera etc., and the definition of these rest raw material varies in different periods and countries. Most of these underutilized parts are wasted or turned to low value silage for animal feed, and only 10% are used in high value product like human food, health food and pharmaceuticals (Ottesen et al., 2016)

Although 72% of the earth surface is covered by water, only 6.5% of the protein sources for human consumption comes from the marine bioresources (Harvest, 2016). The growing population and positive attitudes towards consumption of seafood in the last few years are the main drives for maximum utilization of the whole catch. The quota system that was initiated in the earlier half of the 1980s in Iceland was a symbol of Icelanders' awareness of the limited marine resources and willingness to utilize in a sustainable way (Arnason, 2008). Physiochemical knowledge of these marine species and quality changes during storage is a prevailing key to that door.

Detailed analysis of the components of salmon heads and the analysis of these components storage stability can create a solid database, which is valuable for the farmed Atlantic salmon industry in Iceland. The analysis and collection of data in the database then further provides the prerequisite knowledge for further utilization of these underutilized by-products. These by-products have huge potential for higher reward than fillets after further processing, despite of their low profit nowadays, and studies have shown promising possibilities to acquire lipids, proteins and other valuable resources for food supplement, cosmetics and medicines (Arason, 2003, Rustad, 2003, Rustad et al., 2011). Moreover, quality and stability analysis of these by-products will provide essential information and optimization for their storage and transportation. High-tech jobs will be created subsequently, and this positive feedback circle rolls on. In general, deeper knowledge and sustainable utilization of salmon heads will be beneficial to Iceland economically, environmentally and even politically.

The overall aim of the study was to analyze the physiochemical properties of different parts of Atlantic salmon heads (brains, eyes, gills, tongues, and pectoral fins), and the effect of frozen storage on their quality and stability for 4-month storage.

2 Review of the literature

Salmon is one of the most important commercial fish species in the world and the aquaculture salmon industry in Iceland is in its starting phase. Even though the harvest volume of Atlantic salmon is much lower than for other species in Iceland, Atlantic salmon is a potential product in many fields due to its high nutritional value, high level of industrialization and low level of risk (Harvest, 2016). Most of the side products from salmon industry processing end up as low-value bulk silage or are simply wasted, which calls upon more sufficient utilization. For environmental and economic concerns, it is vital to acquire detailed information of salmon by-products for further improvement of utilization.

2.1 The Atlantic salmon

The Atlantic salmon (*Salmo salar*) as shown in Figure 1~~Figure 1~~, is a species of ray-finned fish in the family Salmonidae and the Atlantic salmon is the largest species in their genus. The coloration of Atlantic salmon alters with age and environment. Young salmon have blue and red spots when they live in fresh water, and they take on a silver-blue sheen at maturity. When they reproduce, males take on a slight green or reddish color. The salmon has a fusiform body, and well-developed teeth, and all fins are bordered with black except the adipose (Bone and Moore, 2008).

Figure 1. Atlantic salmon (*Salmo salar*) (Knepp, 2003)

2.1.1 Biology

Farmed salmon are usually smaller but have more numerous eggs than wild salmon (Jonsson, 1997). Spawning females can lay around 7500 eggs in a controlled environment (NOAA fishery, 2019). Juvenile smolts grow much faster in salt water than in fresh water, but the growth rates depend on season, age, sex and population density (Lucas and Southgate, 2012). After two years at sea, adult salmon have an average length of 71 to 76 cm and a weight of 3.6 to 5.4 kg (NOAA fishery, 2019). Compared with wild-origin salmon, farmed or ranched salmon had smaller-spaced circulation (Friedland et al., 1994).

2.1.2 Farming

Atlantic salmon culture began in the 19th century in the UK in freshwater as a means of stocking waters with parr in order to enhance wild returns for anglers. From the late 1950s, enhancement programs based on hatcheries were established in the United States, Canada, Japan, and the USSR.

There is an on-going debate about land-based and ocean-based marine aquaculture in Iceland nowadays. Land-based marine aquaculture has the advantage of economic feasibility and minimal environmental impact, while ocean-based marine aquaculture can maximally use the resources from the vast ocean (Kerton et al., 2013, Tal et al., 2009). The contemporary technique using floating sea cages originated in Norway in the late 1960s (Knapp et al., 2007). There are mainly two main developmental stages as shown in Figure 2~~Figure 2~~ for ocean-based salmonid farming. Firstly, the alevin (baby salmonid) are hatched from eggs and raised on land in freshwater tanks; secondly, the smolt (juvenile salmon) are transferred to floating sea cages or net pens anchored in sheltered bays or fjords along a coast when they are 12 to 18 months old (The Fish site, 2019).

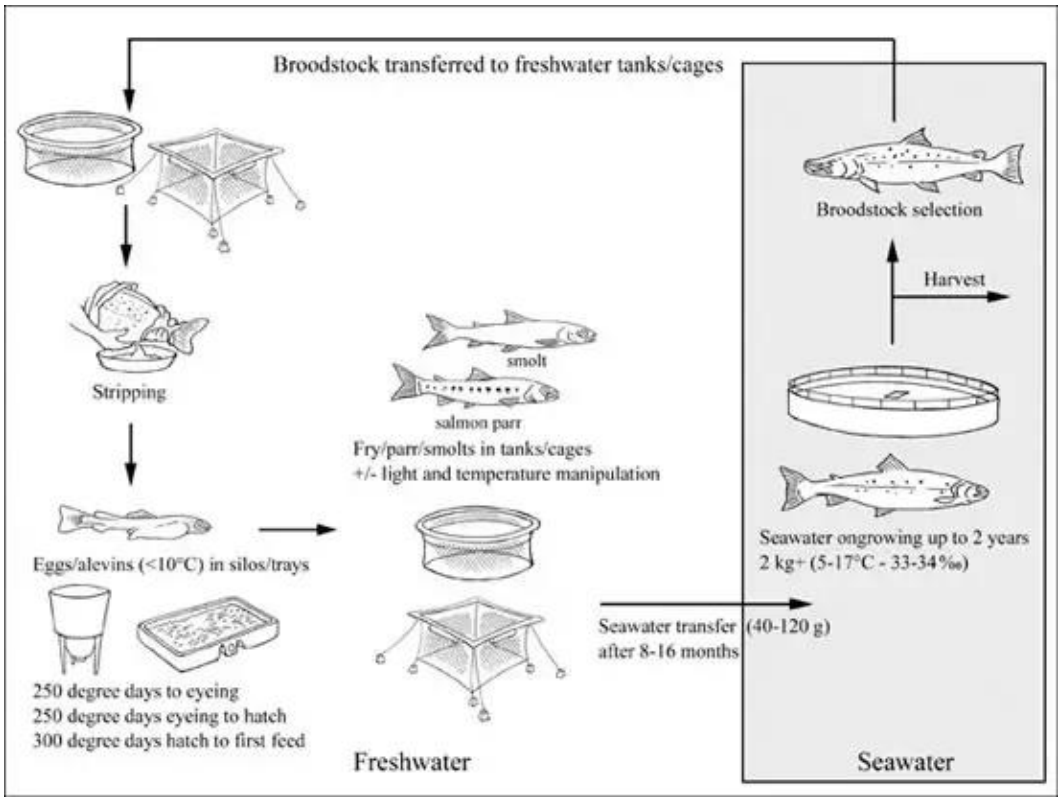

Figure 2 Production cycle of farmed Salmo salar (The Fish site, 2019)

The open net cages of salmonid farming could lower the production costs compared to recirculating or closed systems, but no effective barrier are provided to avoid the access of diseases and wastes in the ambient coastal wasters (Roberts and Shepherd, 1974).

2.1.3 Harvest

Harvesting methods vary in different countries, but salmon are usually starved for up to 3 days prior to slaughtering (Willoughby, 1999). The main aim is to keep the stress to a minimum in the live salmon until being slaughtered to obtain maximum quality. The old way to harvest included transferring the salmon into carbon dioxide saturated water by sweep net before killing, then they are bled by cutting the gill arches and merged to iced water immediately to minimize scale loss (Willoughby, 1999).

Wet-well ships are often used to transport live salmon to the processing plant in modern days. This more humane processing could lower rigor-induced quality deterioration if combined with electrical stunning and bleeding (FHF, 2019).

2.2 Composition and utilization

2.2.1 Water content

According to Bechtel, (2003) the water content in the whole fish of pink salmon (*Oncorhynchus gorbuscha*) is 71.7%, and it is significantly higher in the viscera which is 81.2% (Bechtel, 2003). In Atlantic salmon, the water content in the flesh is 64%-72% and it decreases from the tail to the head (Aursand et al., 2008). It is worth noting that the water content can also change with the storage temperature and may drips out if the temperature becomes too high. But there is about 5%-15% of highly immobilized water in fish that does not freeze even when the temperature is lower than -40°C (Murray and Burt, 2001). Water binding of the muscle is mostly due to the water interactions with actin and myosin, and the water content and distribution in the fish flesh is vital considering the muscle quality. Overall, not only the water content, but also the water distribution and characteristics effects the stability, palatability and overall quality of the muscle. Meanwhile, no significant changes of water content were observed in farmed *Salmo salar* fillet during 14 days iced storage (Hultmann and Rustad, 2002).

2.2.2 Protein content

Proteins for human consumption are constructed out of 20 different amino acids and food usually contains many different types of proteins. Nine types of amino acids are essential amino acids (ten for infants) that humans must acquire from food, and there are four levels of protein structure (Damodaran and Parkin, 2017).

Proteins in fish are high in nutrition, easy to digest and contain all nine essential amino acids (Halver, 2013). However, the protein amount changes because of different feedings, farming methods, gender and life periods. The protein content of whole Atlantic salmon fillets is 18.6%-20.9% (Isaksson et al., 1995), and the protein content decreases all the way from egg spawning (60.1%) to growing up to 1500 g (48.9%) (Shearer et al., 1994). In addition to the high nutritional value, fish proteins may also exhibit bioactive functions, such as antioxidative, antihypertensive, antithrombotic and immunomodulatory properties (Kim and Mendis, 2006a, Rustad et al., 2011). During 11-day iced storage, the total amount of extractable proteins of farmed *Salmo salar* fillet increased at first and then reduced (Hultmann and Rustad, 2002).

2.2.3 Lipid content

Fish and fish lipid products have been shown to be beneficial for human health and the global consumption is growing rapidly (Tacon and Metian, 2008). Many studies show the positive effects of fish intake when it comes to cardiac function, hemodynamics and arterial endothelial function (Caygill et al., 1996, Kris-Etherton et al., 2002, Zhang et al., 1999). The main marine lipids are long chain omega-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid C20:5 n-3 (EPA) and

docosahexaenoic acid C22:6 n-3 (DHA), which are essential for brain formation during pregnancy (Murray and Burt, 2001).

Atlantic salmon is a fatty fish species, and the lipid content varies among its different life stages, feed and farming methods. The lipid content increases as the fish grows from a fertilized egg until they are big enough to hunt in fresh water. For the harvested adult fillet, the mean lipid content varies with position from 2.37%- 18.55% out of wet weight. Thereof are the dorsal and belly parts generally fattier than the other parts (Katikou et al., 2001). The concentrations of DHA and EPA (per 100 g sample) are much higher in the red muscle (DHA, 2.2 g; EPA, 1.5 g) and belly flap (DHA, 1.9 g; EPA, 1.5 g) compared to white muscle (DHA, 0.8 g; EPA, 0.6 g) (Polvi and Ackman, 1992, Aursand et al., 1994). The content of free fatty acid of were observed increasing 3-6 fold from the mixed heads and viscera from *Oncorhynchus gorbuscha* during the 4-day storage at 6°C, but there was no significant change of long-chain omega-3 fatty acids in the same storage condition (Wu and Bechtel, 2008).

2.3 Utilization of by-products

The definition of by-products varies between different fish species, processing methods and how people perceive it in different countries. Fish by-products from processing are mainly generated in two main stages, and the first stage is the handling after catching (discards) and the second one is unutilized materials during processing from raw fish to product (Meldstad, 2015). Nowadays unutilized by-products of fish from the industry can reach up to 50% (Guérard et al., 2005), and there is still lack of knowledge in how to process or store salmon by-products to maintain a high quality in them (Wu and Bechtel, 2008).

In Iceland, the total quantity of marine by-products of all species from the fish industry was 379,000 tons in 2013 and 52,000 tons thereof were utilized, but only 10% out of these were used for human consumption (Ottesen and al, 2016). Norway is in a leading position for utilizing salmon by-products and up to 86% out of 1,301 tons from the salmon industry was properly used in 2013 (Ottesen and al, 2016).

2.3.1 Lipid

Currently, high quality oil could be extracted from fresh fish by-products and oil remains to be one of the most important products from the rest raw materials.

The traditional way to produce crude fish oil includes heating, pressing, and centrifugation. Other new technologies involved in extraction process such as tricanters are able to separate different phases, avoid oxygen and enzymes from crude oil, which offers more predictable properties and lowers the cost for conservation (Rustad et al., 2011).

2.3.2 Protein

Different types of protein products can be obtained from salmon by-products, for example mince and surimi from backbones and cutoffs, gelatin and collagen from heads and skins. The main obstacle for producing a mince protein product is the inconsistency in availability and heterogeneity of the raw materials. However, and there will be more market possibilities if these quality problems are solved properly (Morrissey and Sylvia, 2004).

Fish protein isolates (FPI) are one kind of concentrated product with at least 90% dry matter and is usually processed by pH shifting. New technologies like isoelectric processing and enzymatic hydrolysis are often used for FPI production instead of chemical hydrolysis (Kim and Park, 2007).

Fish protein hydrolysates (FPH) not only have high nutrition value, but they also have excellent properties like excellent water-holding capacity, gelling, foaming and emulsification properties (Kristinsson, 2007). FPI may also show bioactive properties like antioxidative, antihypertensive, antithrombotic and immunomodulatory activities (Kim and Mendis, 2006a). Usually a bitter taste will follow products treated with enzymatic hydrolysis, but the bitterness is related to the degree of hydrolysis and the average hydrophobicity of the peptide (Shahidi, 1994). Several different methods are used to remove this undesirable taste, such as removing the gall bladder before processing and using black beans to bind bile acids (Dauksas et al., 2004, Kahlon and Woodruff, 2002, Thorkelsson and Kristinsson, 2009).

Gelatin and collagen from fish are mainly produced from the skin and bones and the market proportion is increasing. One of the biggest advantages of using fish gelatin or collagen in food is the high compatibility with Muslim and Jewish populations, and other people who avoid products from pork sources (Arason, 2003). The use of fish gelatins also lower food risks like bovine spongiform encephalopathy, compared to the ones obtained from bovine. Gelatins are also widely used in pharmaceuticals, cosmetics, production of photographic films and even bullets (Kim and Mendis, 2006b). However, it is highly urged to produce high and stable quality gelatin at low costs, and better methodology, together with mechanization could be a future solution (Rustad, 2003).

2.3.3 Enzymes

The internal organs of fish are rich sources for a wide range of enzymes. Due to the high pressure and low temperature environment in which the fish live in enzymes in marine fish exhibit high catalytic activities at rather low concentrations (Kim and Mendis, 2006b). These excellent properties are beneficial in many processing operations, such as pharmaceutical, cosmetic and hygienic research fields (Bjarnason, 2001).

2.3.4 Ash and others

Fish by-products have large varieties of minerals including calcium, zinc, copper etc. and the ash content may increase during different industrial processing like drying or canning because of the loss of moisture. Calcium is the richest fraction which makes up 60-70% in bones. Hydroxyapatite ($Ca_5(PO_4)_3OH$) from fish bones is also of alternative interest because of its high bio-compatible in the human body as a substitution for bone (Ferraro et al., 2010). Furthermore, salmon blood is sometimes used as a coloring agent for both animal and human consumption.

In general, fish by-products contain valuable lipids, proteins and other compounds like enzymes and minerals. With deeper research and advanced technologies, the potential value from the by-products can be revealed, and might become even higher for the by-products than for the fillets in the near future.

2.4 Market situation and possibility

The history of using by-products from the fish industry is long and several products made from different raw materials are available in Scandinavian markets.

Heads and trimmings are usually not consumed directly by humans and mostly go to the feed or fertilizer industry. Cod tongues and cheeks are considered as delicacies in Iceland and they are also salted and exported to countries like Spain and Portugal. In Iceland, the yield of the tongue was about 3% of the head weight by machine cutting (Rustad, 2003). In 2001, 11,432 tons of dried cod heads from Iceland were exported to Nigeria as a protein source for the local people and brought 25 million USD to the Icelandic economy (Arason, 2003). In Iceland, the cheap geothermal resources for heating also granted these products more edges in price competition.

Leather shoes, clothing and bags from fish skin have been popular in Scandinavia for centuries. Nowadays, gelatin from fish is also an alternative ingredient for the food industry because of its unique properties and disease transfer absence of mammalian bovine spongiform encephalopathy (BSE) and similar conditions. Collagen from skin is widely used in beauty cosmetics and pharmaceuticals, such as a replacement for scalded skin. In Iceland, dried or frozen fish skin are also exported to countries like Spain and Canada.

The demand for smoked and canned roe is high and recently the main sources in Iceland are from cod, pollock and haddock. Because of the life stage, roes are only harvestable in certain periods annually, which results in the high and increasing price. One of the most successful products out of smoked roe is “caviar”, which is also popular in Asian countries like Japan. The total export quantity of Icelandic roes was 6,165 tons valued to 10.4 million USD in 2001 (Arason, 2003).

2.5 Research objective

The main objective of this research was to analyze the composition of different parts of Atlantic salmon heads and to investigate how the composition would change with prolonged frozen storage time. Brain, tongue, eyes, fins and gills were analyzed individually and the whole head was considered for collagen extraction. Knowledge of the chemical composition and quality changes is crucial for further research and industrial applications. This study will provide a ‘value map’ for Atlantic salmon heads, which may spark ideas of possible ways to increase the value of by-products from Atlantic salmon heads.

3 Materials and methods

3.1 Materials and treatment

There were two batches of farmed Atlantic salmon (*Salmo salar*) heads used in this study supplied by the same land-based farming facilities of Samherji hf. The first batch from October 2018 was for chemical composition and storage stability analysis, and the second batch from March 2019 was for collagen extraction from the whole heads. The salmon roes were bought from Stofnfiskur and hatched in Ölfus. Then the fish grew up in the farming plant in Öxarfjörður and were slaughtered and headed in the same plant when they were 3.5-4.0 kg. The processing flowchart of these two batches of salmon heads are shown in Figure 3~~Figure 3~~.

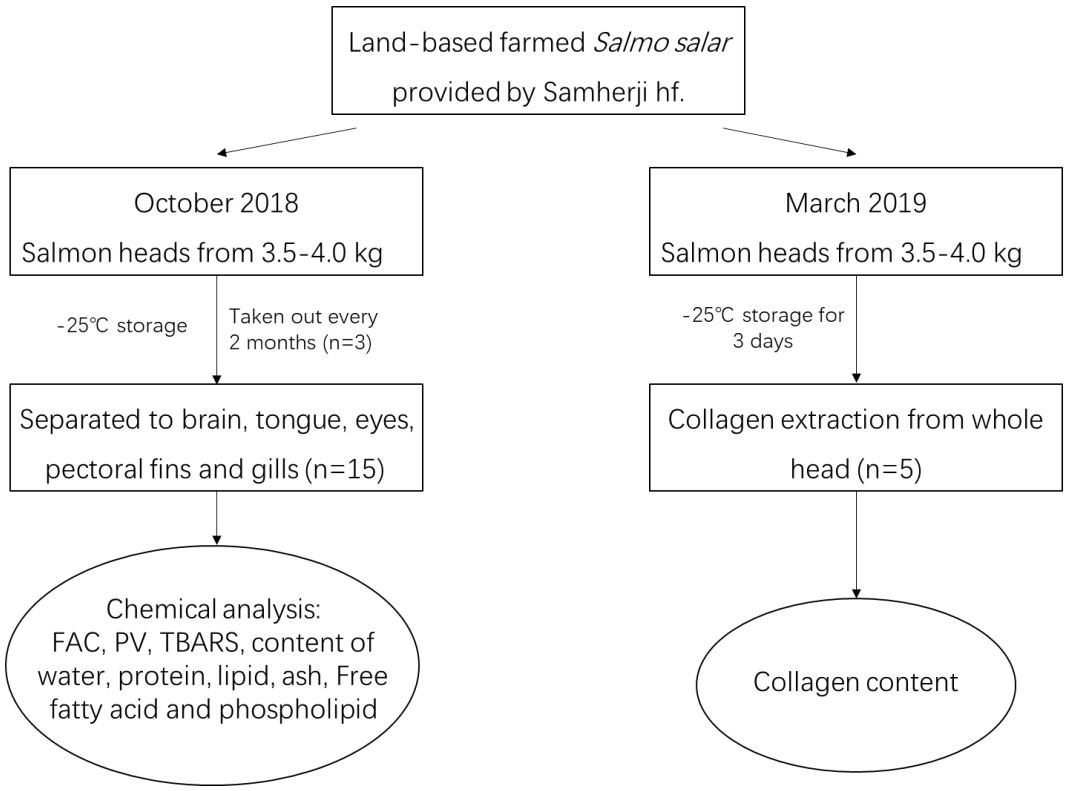

Figure 3 Processing flowchart of two batches of salmon heads in this study

The first batch of 120 fish heads were sent to Matís by Eðalfiskur ehf.in polystyrene boxes with ice slush on October 16th, 2018. These fish heads were immediately transferred to ice-free blue polyethylene bags. Each bag, containing 5 randomly assigned heads was sealed as shown in Figure 4~~Figure 3~~ and put in marked cardboard boxes. Then the boxes were put into a blast chiller (ilsa-ABV4043, Italy) for 4 hours and stored in a -25°C freezer.

Figure 4 Salmon heads arrived and repacked on Oct 16th, 2018

Samplings were performed after 0, 2 and 4 months of frozen storage of 15 heads each time, divided into three sample replicates, each containing 5 heads. Samples were thawed at 0°C for 10 hours prior to separation of the individual parts of each head. The heads were then cut by hand and separated into brains, eyes, gills, tongues and pectoral fins (*hereafter referred to as fins)* for all batches as shown in Figure 5~~Figure 4~~.

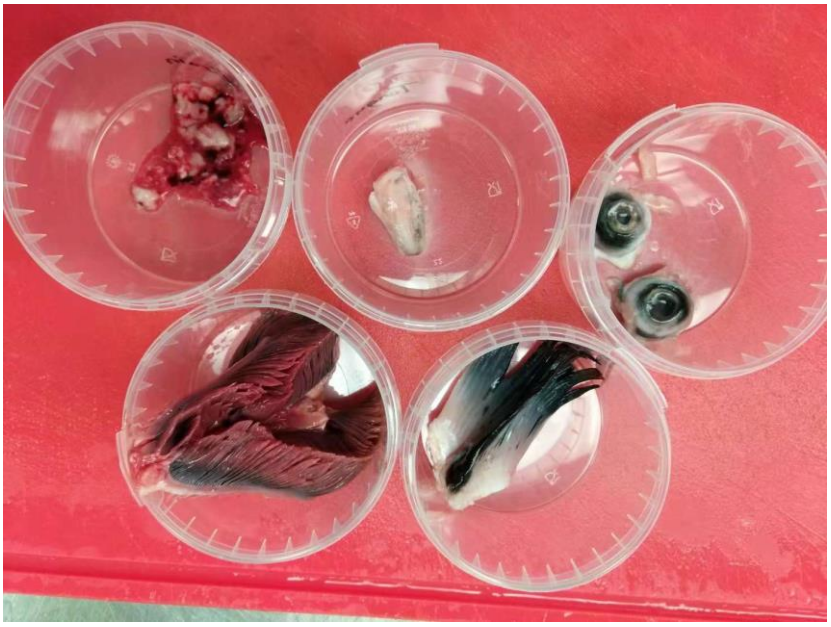

Figure 5 Brain, tongue, eyes, gills and pectoral fins separated from salmon head

Right after the separation, eyes, fins, gills, and tongues were minced individually in a blender (Braun Combimax-600, Germany) for 5 minutes at speed position 14 (no-load speed 1850-2000 rpm) as shown in Figure 6~~Figure 5~~. However, the brain samples were minced in another blender (Bamix M-160, Switzerland) for 30 seconds at speed position 1 (speed 10,000 rpm) because the amount of brain was too small to be fully minced in the former mincer. Samples were stored at -80°C prior to analysis.

Figure 6 Samples to be minced in blender

3.2 Physicochemical analysis

3.2.1 Yield analysis

The heads (15 heads per sampling point) and each part (eyes, brain, gills, fins and tongue) were to calculate the yield of each part of the head according to the following equation:

$$\textbf{\textit{Yield}}\ (\%) = \frac{g\ individual\ part}{g\ whole\ head} \times 100$$

3.2.2 Water content analysis

The water content of the salmon eyes, brains, gills, tongues, and pectoral fins was analyzed in hot box oven (Gallenkamp size 2, Netherland) at 105°C according to the ISO 6494 (1999) method. All chemical analyses were performed on three sample replicates, each replicate containing pooled parts from 5 randomly assigned salmon heads.

3.2.3 Protein analysis

The protein content of the individual parts of the heads was analyzed using the Kjeldahl method as described in ISO 5983-2:2005.

3.2.4 Ash analysis

The ash content was analyzed using the method described in ISO 5984-2002 (E) and mineral analysis was performed according to NMKL 186 (2007).

3.2.5 Lipid analysis

Lipids were extracted each one per sample based on the (Bligh and Dyer, 1959) method. The chloroform phase was used further for determination of the total lipid content, phospholipid content (PL), fatty acid composition (FAC), free fatty acid (FFA) content and primary and secondary oxidation. The lipid content was presented as grams lipid/100 g sample

3.2.6 Fatty acid composition

Fatty acid methyl esters (FAME) are separated on a Varian 3900 GC equipped with a fused silica capillary column (HP-88,100 m x 0.25 mm x 0.20 µm film), split injector and flame ionization detector fitted with Galaxie Chromatography Data System, Version 1.9.3.2 software. The oven is programmed as follows: 100°C for 4 min, then raised to 240°C at 3°C/min and held at this temperature for 15 min. Injector and detector temperature are 225°C and 285°C, respectively. Helium is used as a carrier gas at the column flow 0.8 mL/min; split ratio, 200:1. The Program is based on AOAC 996.06.

3.2.7 Free fatty acid analysis

The free fatty acid (FFAs) content was analyzed from each lipid extract as duplicate, determined by the method of (Lowry and Tinsley, 1976) with modifications as described by (Bernárdez et al., 2005), from the lipid extractions provided by the (Bligh and Dyer, 1959) method, as described earlier. The results were shown as g FFA/100 g lipids.

3.2.8 Phospholipid

The phospholipid content was analyzed from each lipid extraction as duplicate, using a colorimetric method, based on the formation of a complex between phospholipids and ammonium ferrothiocyantate (Stewart, 1980).The results were presented as g phospholipid /100 g lipids.

3.2.9 PV analysis

The peroxide value (PV) was measured in triplicate per sample, using a ferric thiocyanate method (Chapman and Mackay, 1949) and the absorbance of the sample product was read at 500 nm by an Epoch microplate spectrophotometer (BioTek instruments, U.S). The results were presented as mmol/kg lipid.

3.2.10 TBARS analysis

Thiobarbituric acid reactive substances (TBARS) analysis was performed in triplicate per sample based on the method of Folin-Phenol reagent (Lowry et al., 1951) with one change. The supernatant of sample used for analysis was 300 µL rather than 500 µL. The results were presented as µmol/kg samples.

3.3 Collagen extraction

The salmon heads used for the collagen extraction arrived in polystyrene boxes filled with ice on March 15th, 2019 and these fish heads were provided by the same company as mentioned above. Then the samples were transferred with the original boxes to a -25°C freezer directly for storage. The samples

were taken out from the -25°C freezer on March 18th, 2019 and were saw-separated geometrically into eight parts as shown in Figure 7~~Figure 6~~.

Figure 7 Saw-separated salmon heads for collagen extraction

Samples were weighed before pre-washing, then cold tap water was used to rinse the slime and blood away. Then rinsed samples were sieved, then mixed with 0.1 M NaOH solution at the weight ratio of 1:3. The pretreatment took place overnight in a refrigerating room at 0°C. The next day, were the samples taken out of the NaOH solution and rinsed in cold tap water until the pH of the rinsed water was between 6 and 8. Then the samples were sieved and mixed with 10% ethanol solution at the weight ratio of 1:3. This fat removing process took place overnight in a 0°C cooler. The next day, samples were taken out of the ethanol solution and rinsed in cold tap water until the pH of the rinsed water was between 6 and 8. Then samples were sieved and mixed with 0.6 M HCl solution at the weight ratio of 1:3. This demineralization process took place overnight in a 0°C cooler. The next day, samples were taken out of the HCl solution and rinsed in cold tap water until the pH of rinsed water was again between 6 and 8. Then samples were sieved and mixed with 0.5 M acetic acid solution at the weight ratio of 1:3. This extraction process took place overnight in a 0°C cooler. The next day, samples were taken out of the acetic acid solution and rinsed in cold tap water until the pH of the rinsed water was between 6 and 8. Then samples were sieved and mixed with distilled water at the weight ratio of 1:3. This heating process took place for 12 hours in an incubator shaker (New Brunswick Innova 4400, USA) at 80 rpm and 45°C. The next day, samples were taken out of the incubator and the liquid was separated from the solid by a sieve (20 meshes per cm^2). Then the liquid was sent to freeze drying, which lasted for 3-4 days depending on the amount of samples (Poranen, 2019).

The whole extraction process including freeze drying took about ten days to perform, and the flow chart over the process can be seen in Figure 8~~Figure 8~~.

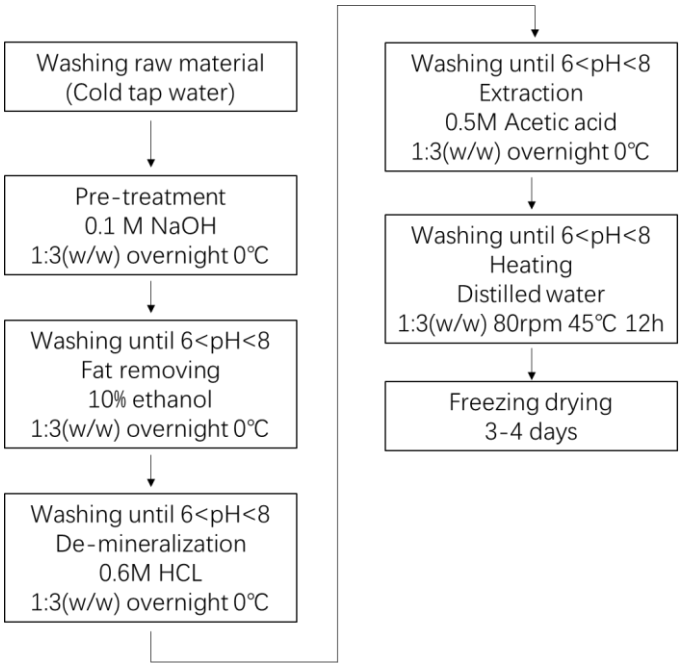

Figure 8 Flow chart of collagen extraction (Poranen, 2019)

3.4 Data analysis

Microsoft 365 Excel (Redmond, USA) was used to calculate means and to create graphs and IBM SPSS 22.0 (Armonk, USA) was used for statistical analysis. Values were presented as mean ± standard deviation (SD) and results were trusted with a 95% significance level ($p<0.05$) for one-way ANOVA test.

4 Results and discussion

4.1 Chemical composition and storage stability

4.1.1 Yield

The yield of the brain, tongue, eyes, gills and fins as a percentage of the whole head as affected by frozen storage time is shown in Figure 9~~Figure 9~~.

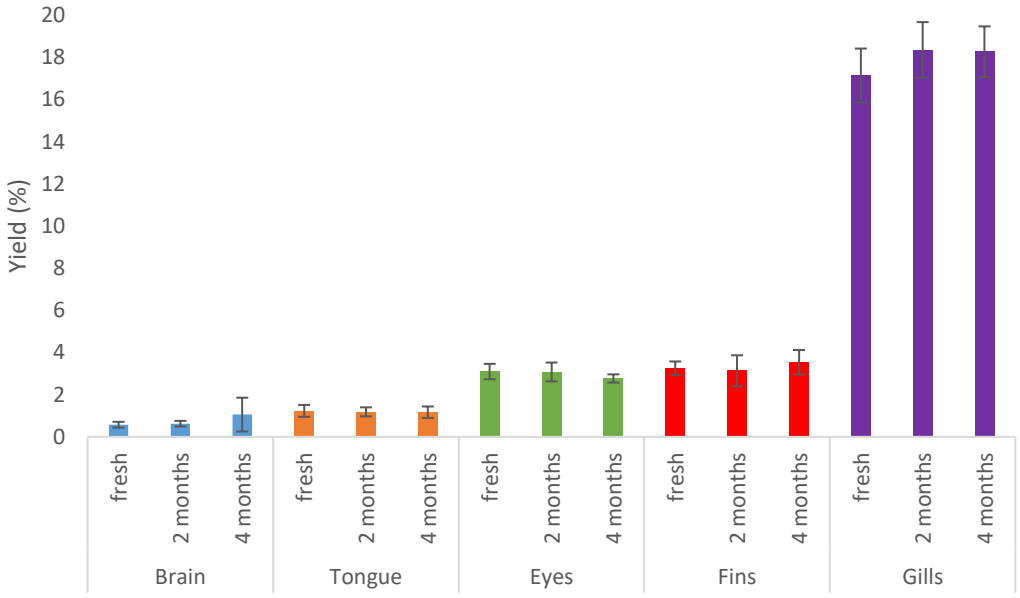

Figure 9 Yield (%) of the five parts from the salmon heads during 4-months of frozen storage (n=15)

The gills had the highest yield of *17.1±1.3*% in the fresh salmon heads among the other parts, while the brain had the lowest yield of *0.6±0.1*% of the whole head. The brain of the Atlantic salmon was a very small part inside the frontal head, which was difficult to approach. Small samples also adhered to the separating tool, which could cause some fluctuations in the sampling yield of the brain. Connective tissue and nerves attached to the eyes could be one of the interference factors for their yield deviation. Because of potential nonuniform industrial processing or intra-species combat, some of the fish only had one fin, which could be a possible contribution to the standard deviation for the fins.

There were slime and melted ice from the samples stuck to the knife and cutting board during the separation, which explains why the sum of the yield values do not add up to 100%. Any other parts from the head, such as collar and skull, were classified as “other” rest raw material and accounted for *71.7±1.4*% of the fresh samples. This big part of the head was not used for further analyses in the current project.

The yield of these five parts did not change significantly during the four months. The tongue and fins were more stable than the others and the obscure change of brain, eyes and gills could be due to the individual difference. Considering the yield and simplicity of separation, tongue, eyes, fins and gills have great potential to be industrially utilized in large scale.

4.1.2 Water content

The water content of the salmon brain, tongue, eyes, gills and fins as a percentage of 100 g of sample is shown in Figure 10~~Figure 10~~.

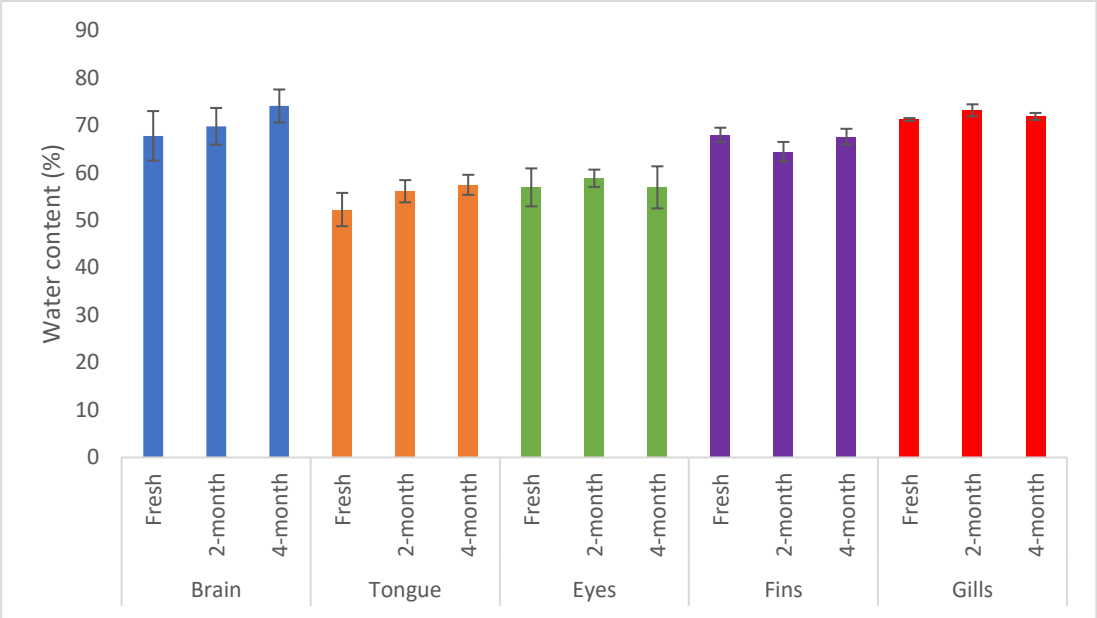

Figure 10 Water content (%) of the five parts from the salmon heads during 4-month storage (n=3)

Generally speaking, the water content in the fresh samples had much variation among one another and ranged from *52.2%* to *71.2*%. The gills and brain had higher water content than the other tissues, and the fresh gills contained *71.2±0.3*% water and the fresh brain had *67.8±5.2*% water. The fins followed in water content after gills and brain, while the eyes and tongue had the lowest water content. However, no significant differences were observed in the water content due to the frozen storage in any of the five parts of the head.

Water content can have a high variation between different species (Huss, 1995) as well as different parts of the fish. The water contents of the brain, fins and gills in this research was comparable to the water content in farmed Atlantic salmon fillet muscle (*68.8±2.2*%) (Aursand et al., 2008), while the water contents of the tongue and eyes were a bit lower. The water contents of tongue and gills in this research are lower when compared to the water contents of tongue and gills from 3-4kg wild cod caught in November (Viðarsdóttir, 2018).

4.1.3 Protein content

The protein content of the salmon brain, tongue, eyes, gills and fins as a percentage of 100 g of tissue sample is shown in Figure 11~~Figure 11~~.

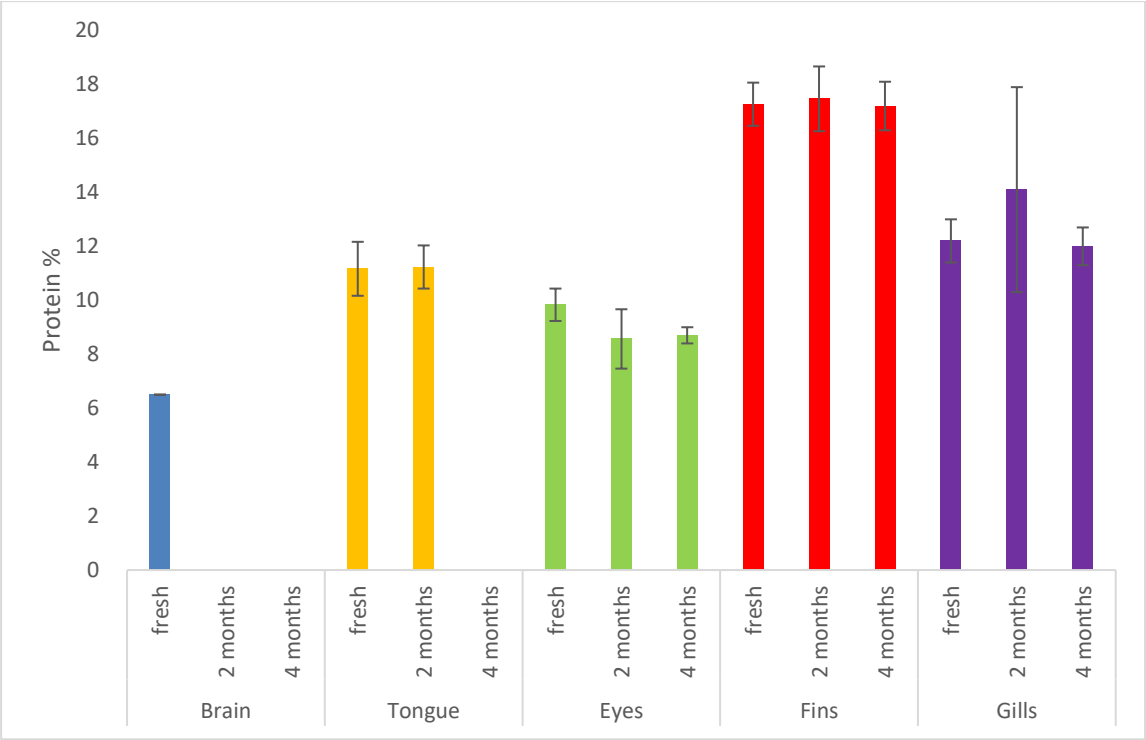

Figure 11 Protein content (%) of five parts from salmon heads in two batches during 2 months (n=3)

For the five analyzed parts from the fresh salmon heads, the protein content varied from *6.5*% to *17.3*%. The fins had the highest content of *17.3±0.8*% and the brain had the lowest content of *6.5±0.0*%. The fresh tongue and gills also had high protein content compared to the fresh eyes. There was no significant differences in the protein content observed for tongue, eyes, fins and gills during these two months of frozen storage, but the amount of water-soluble protein and extractable protein may still decrease during the storage duration (Barraza et al., 2015, Hultmann and Rustad, 2004).

Compared with the protein content (*13.9±1.0*%) of whole heads from pink salmon (*Oncorhynchus gorbuscha*) (Bechtel, 2003) and fillets (*18.2±0.5*%) from farmed Atlantic salmon (*Salmo salar*), fins have higher protein content and would be an interesting alternative protein source for further utilization, not least since it is very easy to remove from the fish. Salmon gills also have high protein content and could be used as protein supplement for silage production, if the unpleasant color and microbial activity could be under control. No significant difference was observed for all the five parts during the four months storage at -25°C.

4.1.4 Ash content

The ash content of the salmon brain, tongue, eyes, gills and fins as a percentage of 100 g of tissue sample is shown in Figure 12~~Figure 12~~.

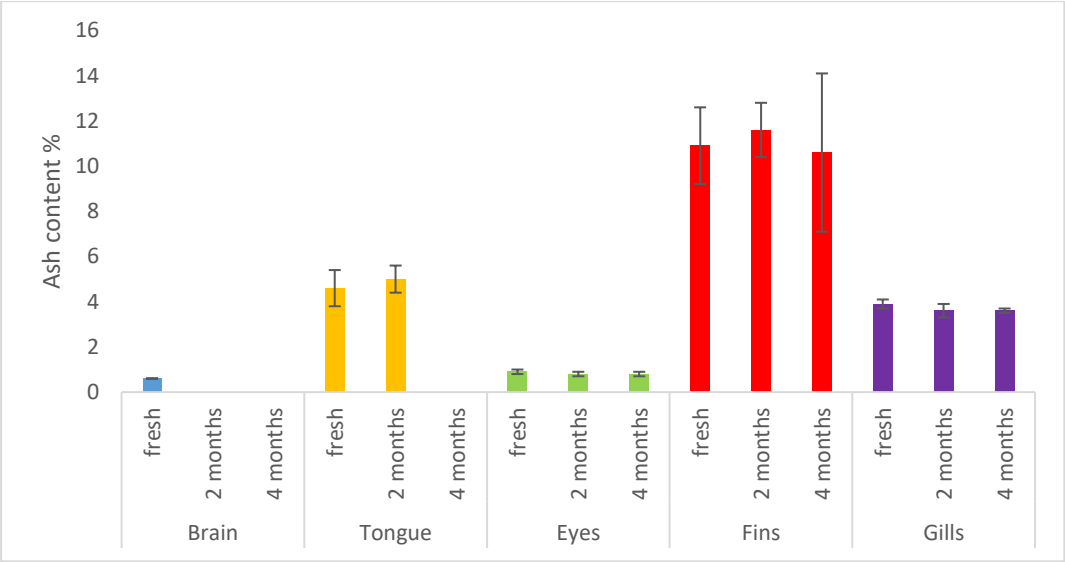

Figure 12 Ash content (%) of five parts from salmon heads in three batches during 4 months (n=3)

The average ash content of the five analyzed parts from fresh salmon heads ranged from *0.6*% to *10.9*%. Compared to the soft tissues, hard tissues like fins, tongue and gills had more bone-like structures and cartilage, which explained the high content of ash. There was no significant difference of ash content observed in the tongue during the first 2-month storage, and the ash content of the eyes, fins and gills were also stable during these 4 months of frozen storage.

Compared to the ash content (*2.6±0.6*%) of whole heads from farmed Atlantic salmon (*Salmo salar*) (Gbogouri et al., 2006) and the ash content (*3.4±0.3*%) of whole head from pink salmon (*Oncorhynchus gorbuscha*) (Bechtel, 2003), had the fins and tongues of the salmon in this study higher ash contents. The ash content of tongue in this research is almost four times higher than the ash content of tongue from wild cod caught in November (Viðarsdóttir, 2018). The fins and tongue could thus be used as potential ash resources for human consumption and feed processing.

4.1.4.1 Mineral content of gills

The mineral elements of iron, zinc, sodium, potassium, calcium and magnesium content of the salmon gills from the fresh samples and samples after 2-month of frozen storage is shown in Table 1~~Table 1~~.

Table 1 Mineral elements of gills from two batches during 2-month frozen storage at -25°C (n=3)

Elements	Fresh	2-month
Iron (mg/kg)	32.70±2.19[a]	35.17±4.10[a]
Zinc (mg/kg)	187.89±15.09[a]	160.84±17.41[a]
Sodium (g/kg)	2.97±0.30[a]	3.10±0.08[a]
Potassium (g/kg)	1.85±0.07[a]	1.77±0.07[a]
Calcium (g/kg)	11.87±1.53[a]	10.59±0.48[a]

Magnesium (g/kg)	0.61±0.05[a]	0.54±0.05[a]

[ab] Different superscript letters at each row indicate significant differences ($p < 0.05$)

The high content of calcium in salmon gills could be the contribution of the cartilage and bony structure. And the calcium content from farmed Atlantic salmon (*Salmo salar*) gills used in this research was significantly higher than the ash content (*3.0±0.44*g/kg) of whole heads from pink salmon (*Oncorhynchus gorbuscha*) (Bechtel, 2003). Gills are relatively easy to separate from salmon heads by hand, mass production for mineral supplement is feasible if machinery with accuracy and gentleness could be applied. Hydroxyapatite has widely been used as biomaterial because of its unique structure and good biocompatibility (Song et al., 2008), and it is also applied in the delivery of protein and gene (Paul and Sharma, 1999, Uskoković and Uskoković, 2011) . Considering the high calcium content and the structure of the salmon gills, trying to extract hydroxyapatite could be an exciting idea. No significant difference was detected for these six analyzed mineral elements during the 2-month frozen storage.

4.1.5 Lipid content

The lipid content of the salmon brain, tongue, eyes, gills and fins as a percentage of 100 g of tissue sample is shown in Figure 13~~Figure 13~~.

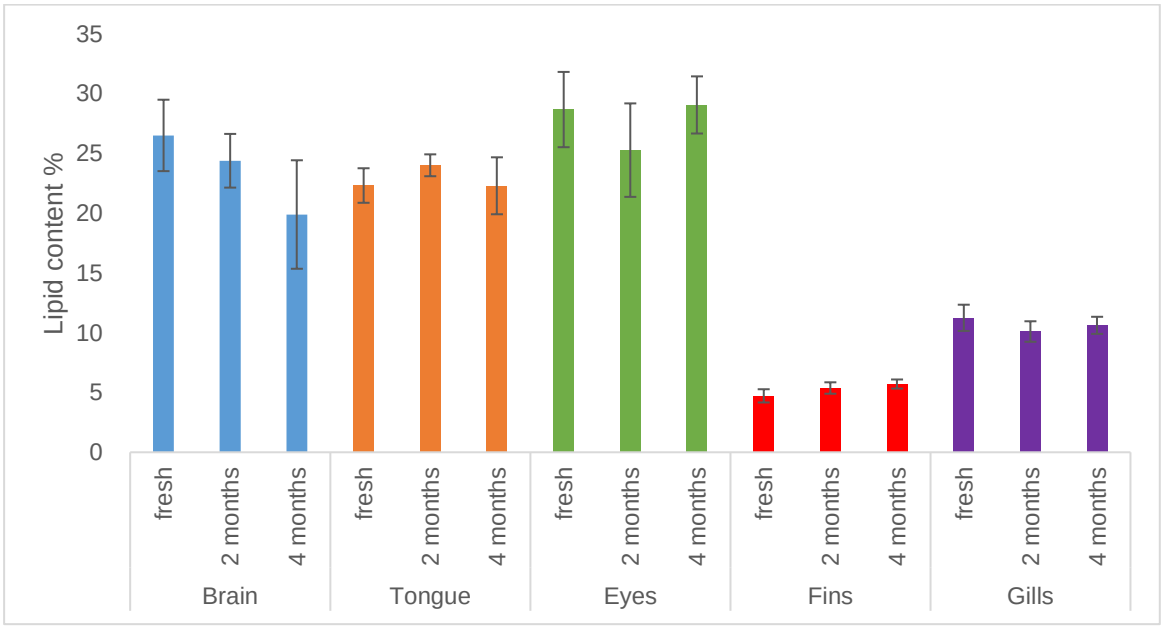

Figure 13 Lipid content (%) of the five analyzed parts from the salmon heads during 4-months storage (n=3)

The lipid content of the five parts from fresh salmon head had a wide range from *4.7*% to *28.7*%. For fresh samples, brain and eyes had the highest lipid content of *26.5±3.0*% and *28.7±3.1*% respectively, and fins had the lowest lipid content of *4.7±0.6*%. The high lipid result of brain was understandable because the oil phase from brain was apparently visible right after separation as shown in Figure 14~~Figure 14~~. Fins and gills had lower lipid content, and interestingly, the lipid extraction from the fins were yellower than the others.

Figure 14 The visible oil phase from sample of brain after separation from head

No significant difference was observed in the brain during the frozen storage. The lipid content of the tongue had a significant increase in the first 2-month storage. The lipid content of eyes decreased significantly from fresh to 2-month storage, and the same with the gills in the first 2-month storage. The lipid content of the fins had a significant increase from 2-month storage to 4-month storage. There is a lack of literature to explain this change, but it is reckoned the lipid content changing has nothing to do with water content since all five parts didn't experience significant change of water content during the frozen storage.

Compared to the lipid content (*2.37%-18.55*%) in harvested Atlantic salmon fillet (Katikou et al., 2001), brain, tongue and eyes from farmed Atlantic salmon used in this research were much fattier. But the results of brain, tongue and eyes agree with the lipid content (*21.5±0.4*%) of the whole head from farmed Atlantic salmon (Gbogouri et al., 2006) and the lipid content (*19.3±0.4*%) of the whole head from commercially farmed Atlantic salmon (Aursand et al., 1994). Since around 72% of the whole head was not chemically analyzed, it could be reckoned that this big part also had high lipid content. Fins are very lean, even compared to the farmed Atlantic salmon head-zone fillets (*11.5±3.1*%), which were frozen and stored at -20℃ for three weeks (Aursand et al., 2008). Compared to the lipid contents of whole head from other species like Alaskan pollock (*Theragra chalcogramma*), Pacific cod (*Gadus macrocephalus*) and Pink salmon (*Oncorhynchus gorbuscha*), which contained *1.2±0.5*%, *0.9±0.3*%, *10.9±0.4*% lipid respectively (Bechtel, 2003), the brain, tongue, and eyes from farmed Atlantic salmon used in this research would thus be good alternative resources for lipid.

4.1.6 Fatty acid composition

The fatty acid composition of the salmon brain, tongue, eyes, gills and fins presented as g per 100 g of lipid is shown in Figure 15~~Figure 15~~. The EPA/DHA ratio of five parts for 2 months storage is shown in Table 2~~Table 3~~. And the data of fatty acid composition of these five parts is shown in Table 3~~Table 4~~ (Appendix B). Tables with detailed values showing information of individual fatty acids from five different parts of salmon heads during the 2-month storage can be found in Appendix C. The FAC was not done

for the four months storage samples because there were no general significant difference in between different parts and neither the same part during frozen storage as well.

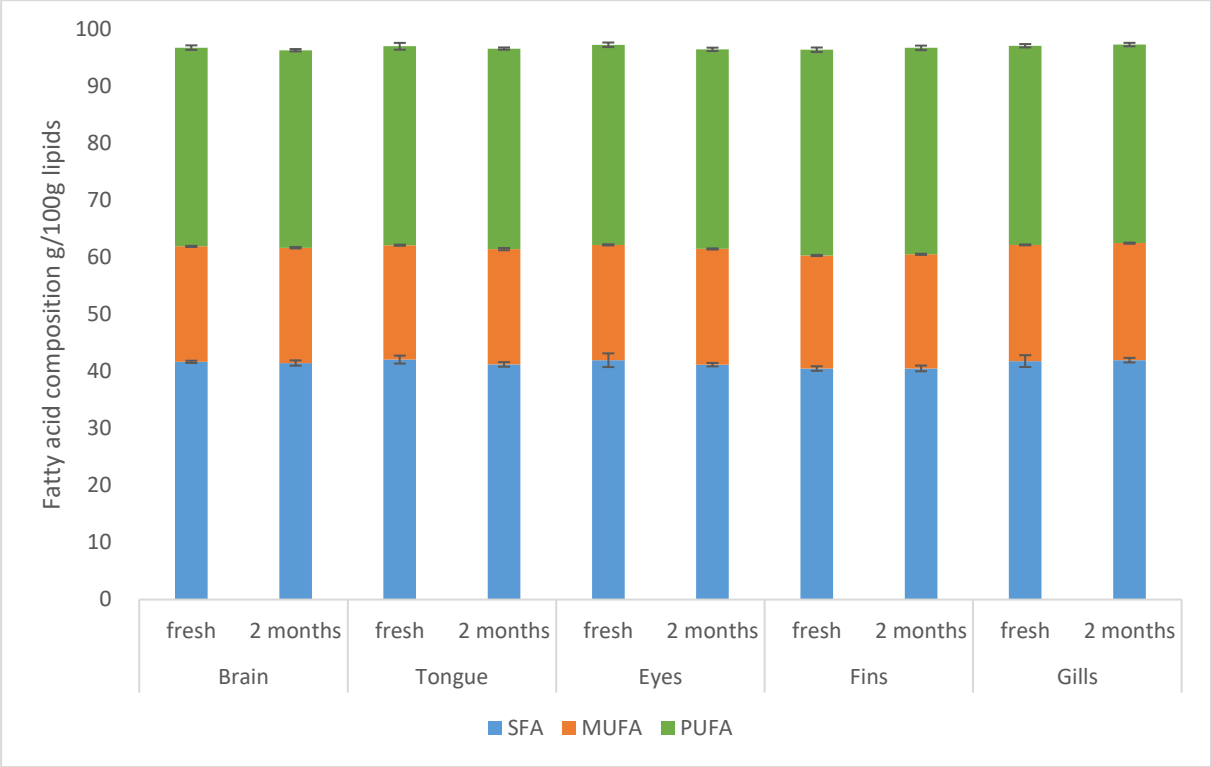

Figure 15 Fatty acid composition of the five parts from salmon head when fresh and following 2-month of frozen storage (n=3)

As we can see from Figure 15~~Figure 15~~, the individual variation in the fatty acid groups of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) was very small between the different parts from the salmon head. For fresh samples, SFA varied between *45.1*% and *48.0*%, and eyes had the highest SFA content of *48.0±0.4*%; MUFA varied between *19.4*% and *20.4*%, and the gills had the highest MUFA content of *20.4±0.1*%; PUFA varied from *19.6*% to *20.1*%, and the eyes had the highest content of *20.1±0.2*%.

The SFA in the brain, tongue and eyes decreased significantly decreasing between the two sampling points: the brain decreased from *47.4±0.5*% to *46.0±0.5*%; the tongue from *47.1±0.5*% to *45.3±0.6*%; and the eyes from *48.0±0.4*% to *45.6±0.5*%. No significant difference was observed in the SFA of the fins and gills during these two months.

The MUFA content increased significantly in the fins and gills during the two months: the fins increased from *19.8±0.1*% to *20.0±0.1*%; and the gill increased from *20.4±0.1*% to *20.5±0.1*%. On the contrary, the MUFA of the brain, tongue and eyes had no significant difference during the storage.

The PUFA content of the fresh batch varied from *19.6±0.2*% to *20.1±0.2*% and all five parts of the head were quite stable during these two months without significant difference detected.

SFA, MUFA, and PUFA are quite evenly distributed in these five different parts. However, the results do not stand in agreement with the fatty acid contents of whole heads from farmed Atlantic salmon (SFA

24.7–27.3%; MUFA *39.9–40.8*%; PUFA *32.3–35.4*%), especially the content of C18:0 and C18:1n9 (Gbogouri et al., 2006). This could be explained by the absence of the other parts of the head, which were not included in the analysis, but this part makes up almost 72% weight of the whole head. It could be reckoned that this remaining material might have higher a percentage of MUFA and PUFA than the five analyzed parts in this research, compared to other results from the whole head of farmed Atlantic salmon (Aursand et al., 1994).

Table 2 EPA/DHA ratio in five parts from salmon head during 2-month storage at -25°C (n=3)

Material	Fresh	2-month
Brain	1.1±0.0[a]	1.2±0.0[a]
Tongue	1.1±0.0[a]	1.2±0.0[b]
Eyes	1.2±0.0[a]	1.3±0.1[a]
Fins	0.8±0.0[a]	0.8±0.0[a]
Gills	1.0±0.0[a]	1.0±0.0[a]

[abc] Different superscript letters at each row indicate significant differences ($p < 0.05$)

As we can see from Table 2~~Table 2~~, the EPA/DHA ratio for fresh sample varied between 0.8 and 1.2 and fins had the lowest ratio of *0.8±0.0*. The EPA/DHA ratio of 1:1 was revealed to have the highest expression of enzymes such as AMPK and PPARα and have protective effect on liver damage in mice because of high-fat diet (Shang et al., 2017).

Only tongue experienced a significant increase of the EPA/DHA ratio during these two months through ANOVA test, but the change is too small considering the ratio itself. There was no significant change during the two months of storage for the other four parts.

4.1.7 Free fatty acids

The free fatty acid content of the salmon brain, tongue, eyes, gills and fins presented as g per 100 g of lipid is shown in Figure 16~~Figure 16~~.

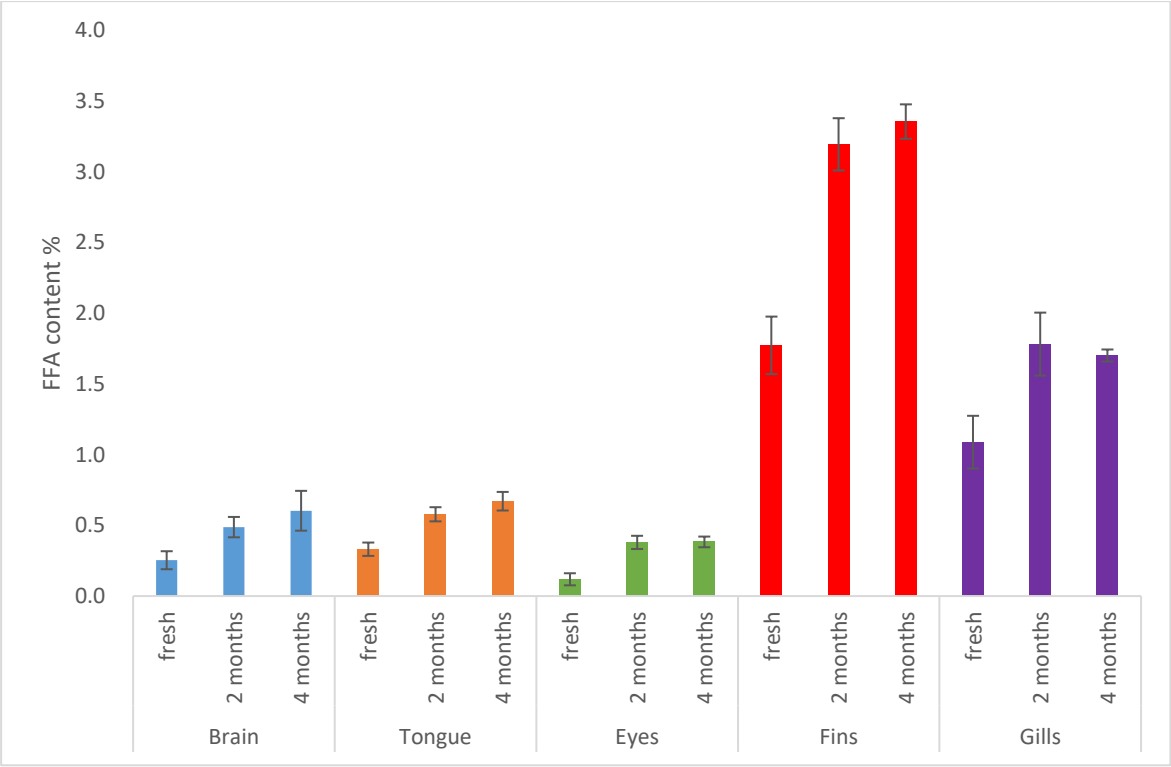

Figure 16 FFA content (g FFA / 100 g lipid) of five parts from salmon heads in three batches during 4 months (n=3)

FFA content for the five parts from the fresh salmon head varied from *0.12* g FFA / 100 g lipid to *1.77* g FFA / 100 g lipid; the eyes had the lowest FFA content of *0.12±0.04* g FFA / 100 g lipid and the fins had the highest FFA content of *1.77±0.20* g FFA / 100 g lipid. Fresh fins and gills had higher FFA values than the other three parts because they are leaner, and this also led to a wider variation in FFA in these tissues during the storage than in the other three oily parts of the head.

Significant increases of FFA content of all the five parts from salmon head were observed during the 4-month frozen storage. This is expected since lipase is still active at low temperature and more FFA will be liberated from ingested triacylglycerols or wax esters with longer storage (Greene and Selivonchick, 1987). For brain, eyes and gills, there was significant difference between the fresh and 2-month sampling points, but no statistically significant difference was observed between the 2-month storage batch and 4-month sampling points. For both tongue and fins, there was significant increase of FFA between fresh, 2-month sampling point and 2-month sampling point and 4-month sampling point by ANOVA test.

As noticed from the data above, the FFA content increased in the first two months and then the accumulation slowed down in the next two months. One possible explanation is that the concentration of reactants was high in the beginning, and the reaction was slower due to the dropping concentration of reactants by the reaction itself (Damodaran and Parkin, 2017).

The FFA contents of fresh fins and gills from farmed Atlantic salmon (*Salmo Salar*) used in this research are similar to the FFA content (*1.45±0.15* g FFA / 100 g lipid) of the whole heads from Pink salmon (*Oncorhynchus gorbuscha*), which were frozen fresh in blast freezer at -30°C overnight; but the

FFA contents from oily parts like brain, tongue and eyes were still lower than the FFA content from the whole head of Pink salmon mentioned above (Bechtel, 2003).

4.1.8 Phospholipid content

The phospholipid (PL) content of the salmon brain, tongue, eyes, gills and fins as a percentage of 100 g of lipid is shown in Figure 17~~Figure 17~~.

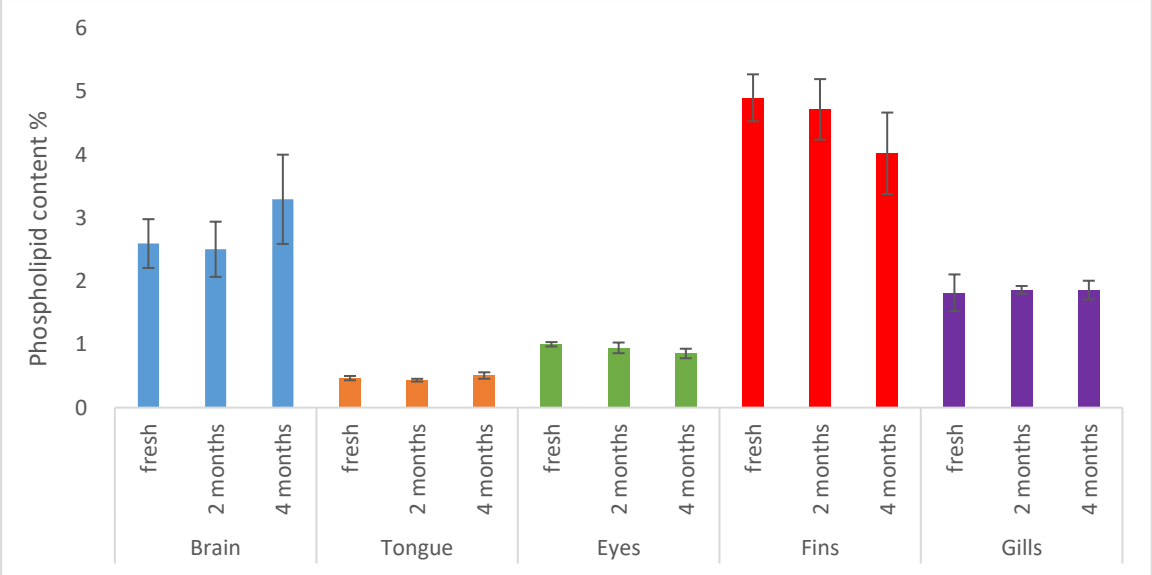

Figure 17 Phospholipid content (g PL/ 100 g lipid) of five parts from salmon heads in three batches during 4 months (n=3)

Phospholipid content of these five analysed parts from fresh salmon head varied between 0.47 g PL /100 g lipid and 4.90 g PL/ 100 g lipid: fins had the highest content of *4.90±0.37* g PL/ 100 g lipid because the total lipid content of fins was the lowest; tongue had the lowest content of *0.47±0.03* g PL/ 100 g lipid. Brain also had high phospholipid content compared to the tongue, eyes and gills.

In general, the phospholipid content of these five parts were quite stable during the 4-month frozen storage and significant difference was observed only in the tongues and fins. Phospholipid content of the tongue increased from *0.43±0.02* g PL/ 100 g lipid to *0.51±0.05* g PL/ 100 g lipid during the last two months. The phospholipid content in the fins decreased from *4.90±0.37* g PL/ 100 g lipid to *4.02±0.64* g PL/ 100 g lipid during these four months, and there was significant difference between the 2-month storage batch and 4-month storage batch. This could be explained by the enzyme destruction of phospholipid during freezing, frozen storage and thawing (Wilson and Rinne, 1976).

4.1.9 Peroxide value

The peroxide value (PV) of the salmon brain, tongue, eyes, fins and gills in unit of µmol/ kg muscle is shown in Figure 18~~Figure 18~~.

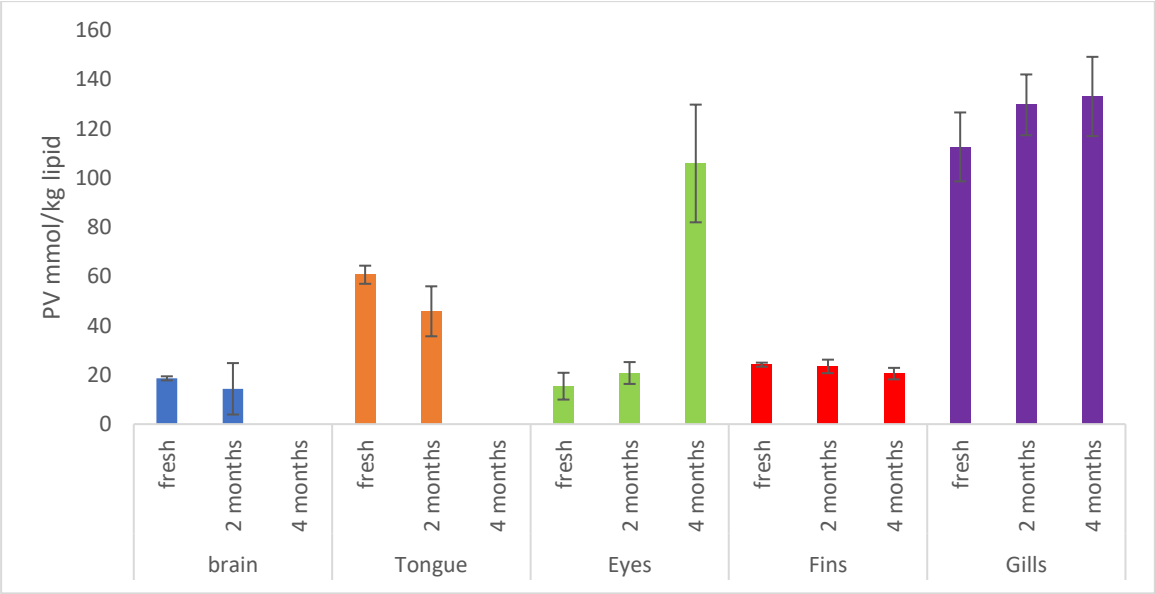

Figure 18 Peroxide value (μmol/ kg muscle) of five parts from salmon heads in three batches during 4 months (n=3)

In the fresh samples, the peroxide value of these five analyzed parts varied widely from *15.4±5.4* μmol/ kg muscle to *112.7±14.0* μmol/ kg muscle. The gills had the highest PV, followed by the tongues. The oily parts, like brain and eyes, had the two lowest PV contents.

There was no significant difference for the brain between the fresh batch and 2-month storage batch. The PV of the tongue decreased significantly in the first 2 months of frozen storage and both values were high, which indicates that the primary oxidation was active during these months. The PV of the eyes did not increase significantly until after 2 months of frozen storage, and the value increased severely from *20.8±4.4* μmol/ kg muscle to *105.9±23.9* μmol/ kg muscle. This indicates that the primary oxidation of the eyes mostly likely happened between the 2-month storage and 4-month storage sampling points. No significant difference was observed for the fins in the first two months but there was a significant decrease from 2-month storage to the 4-month storage sampling point, which means that the primary oxidation of the fins had already happened and really fast even when they were fresh. Noticeable high PV values and a significant increase in the first two months of frozen storage of the gills indicated the primary oxidation of gills was on-going intensively and even stronger during the 4-month frozen storage.

4.1.10 TBARS

The thiobarbituric acid reactive substances (TBARS) of the salmon brain, tongue, eyes, fins and gills in unit of μmol malondialdehyde (MDA)/ kg muscle is shown in Figure 19~~Figure 19~~.

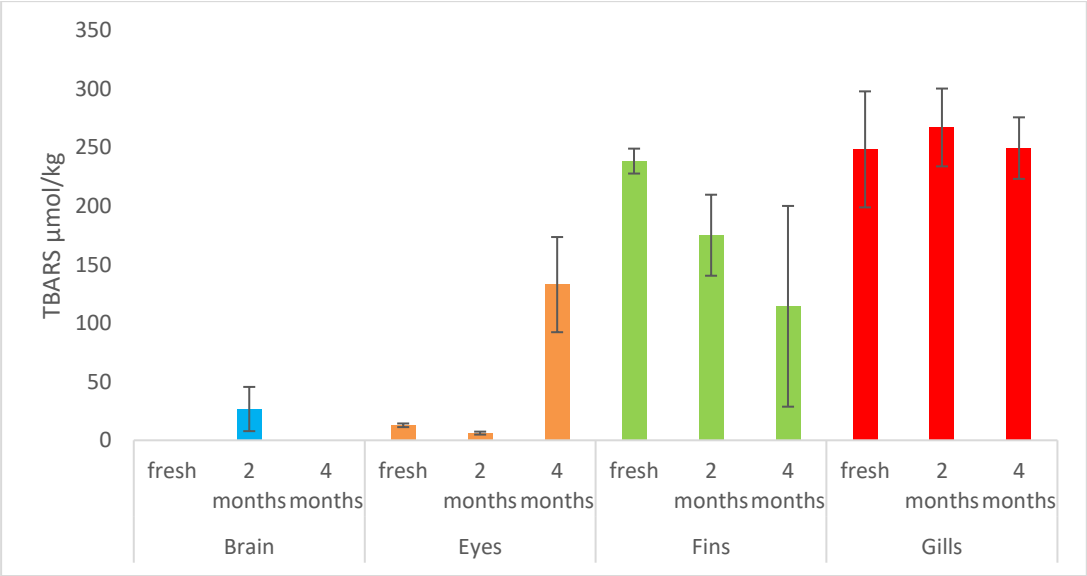

Figure 19 TBARS (µmol MDA/ kg muscle) of five parts from salmon heads in three batches during 4 months (n=3)

Assay of TBARS measures malondialdehyde (MDA) present in the sample muscle. There was a significant decrease of TBARS value in the eye in the first two months of frozen storage, then the value increased strongly to *133.0±40.6* µmol MDA/ kg muscle at 4 months of frozen storage. Combined with the data from the PV of the eyes, this synchronization of peaks in the oxidation products could be explained in a meaningful way: the accumulation of peroxide radicals from the primary lipid oxidation became the reactant of the secondary oxidation (Ladikos and Lougovois, 1990). Comparing the PV and TBARS values of the fins, it could be clearly seen that the major stages of both primary and secondary lipid oxidation took place before the samples were sent to Matís. There was no significant difference in TBARS values observed in the gills during the frozen storage, but the constant high value indicated a constant high level of secondary oxidation. Even though the data from the brain for the fresh and 4-month storage sampling points was missing, it could still be seen that the secondary lipid oxidation of the brain was much slower than in the fins and gills.

4.1.11 Mass balance

The mass balance of products from fresh salmon heads is shown in Figure 20~~Figure 20~~.

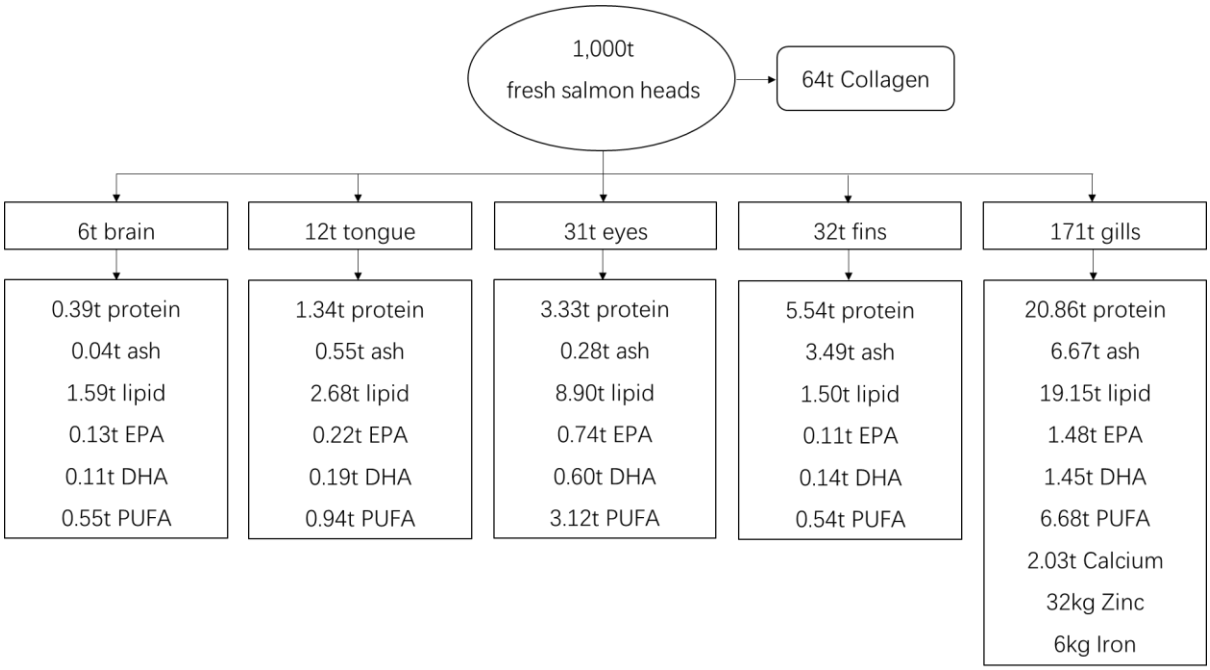

Figure 20 Mass balance of products from 1,000t of fresh salmon head

The yield of brain is the lowest among the five and is not easy to be approached unfrozen. Even though the lipid content of brain is high, it may not be a perfect source for lipid extraction. Among the three oily parts of brain, tongue and eyes, eyes have the highest yield and are easy to be extracted by hand; the lipid content and PUFA content of eyes in general is considerably higher than those three oily parts, and eyes can be an interesting source of EPA and DHA. And the machinery that is used to extrude seeds from cherries can be improved and applied for extraction of salmon eyes in industry (Ottesen et al., 2016). The fins have second highest yield among the five and have excellent protein, ash and phospholipid contents, which grant fins with high value-added possibility. The gills have the highest output of protein, ash, lipid and phospholipid each individually due to its high yield, and if the microbial and enzymatic activity could be controlled, gills will be the perfect sources for general industrial utilization from salmon head.

According to FDA, the dosages of minerals recommended for 4 years of age and older per day are as follow: iron (18 mg) ; zinc (15 mg); sodium (2,400 mg); potassium (3,500 mg); calcium (1,000 mg); magnesium (400 mg) (FDA, 2019). Taking calcium and zinc for example, the calcium extracted from one ton of fresh gills can support about 400 people (4 years and older) for one month; the zinc extracted from one ton of fresh gills can support about 420 people (4 years and older) for one month.

4.2 Collagen

The collagen extracted from whole salmon heads is slightly yellowish as shown in Figure 21~~Figure 21~~ and even though the samples had been pretreated in NaOH solution and the fat had been removed by ethanol solution, the end-products following the freeze-drying still contained oil.

The yield of collagen from the raw whole salmon head was *6.4±0.1*%, lower than the yield of whole head (10.6%-12.3%) from marine cod (*Gadus Morhua*) (Meldstad, 2015), and much lower than the yield

of skin (24.8±0.9%) from farmed Atlantic salmon (Kołodziejska et al., 2008). This is understandable because salmon is not as lean as cod (Viðarsdóttir, 2018) and skin contains more protein due to composition differences in the tissues.

The protein content of the collagen ranged from 51.1% to 76.8%, and this was similar to the protein content (67.7%-71.6%) of FPH, which was enzymatically extracted from whole head of marine cod (Meldstad, 2015).

In general, the extraction of collagen from salmon head pointed out a possible way of utilizing the whole head without further separation, and the yield and protein contents were satisfying compared to marine cod (*Gadus Morhua)* considering the species difference.

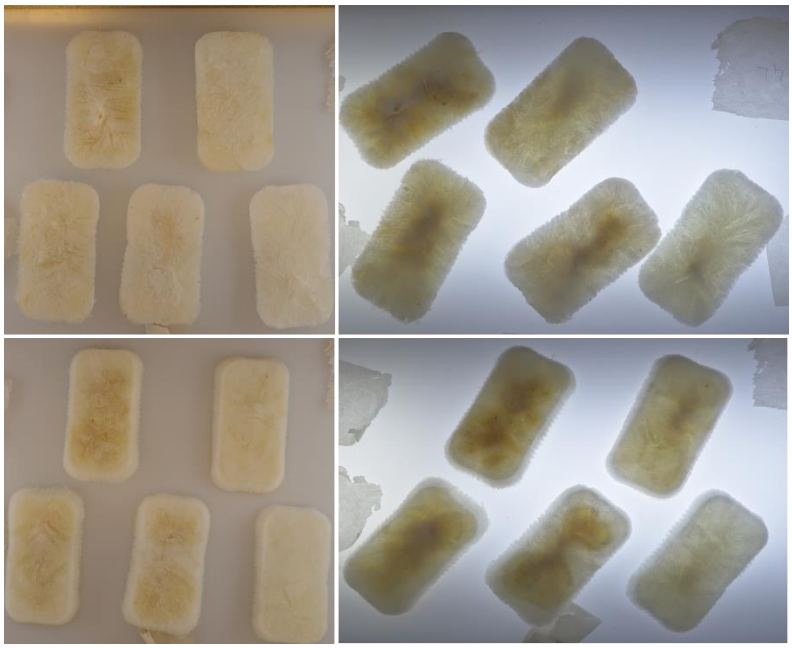

Figure 21 Pictures of collagen extracted from five different heads individually

5 Conclusion

Based on this research, the physiochemical properties of the brain, tongue, eyes, fins, and gills were demonstrated, and the quality and stability affected by frozen storage at -25°C for four months were determined.

The yield had a wide range among those five parts due to the size differences of the heads and the yield changes of the five parts during the 4-month frozen storage were not apparent in general.

The water content of the analyzed five parts from fresh salmon heads had a wide variation from *52*% to *71*%. However, no significant differences in the water content was observed during the frozen storage for all five parts.

The fins had a significant higher protein content than the other four parts and hard tissues like the tongue and gills contained more protein than the brain and eyes because of the higher proportion of muscle and connective tissues included in these parts. Meanwhile, protein contents of all five parts did change significantly during the frozen storage.

The ash content had a wide range from *0.6*% to *10.9*%, and bony tissues such as fins, tongue and gills had higher ash content than the others. No significant change was observed for the ash content of eyes, fins and gills during frozen storage. As for minerals, the gills were rich in calcium and could be a potential source for human usage.

The brain, tongue and eyes were relatively oily compared to the other parts, especially the fins. All of those five parts had significant change of lipid content during the 4-month storage and this could be the result from lipid oxidation and migration.

All five analyzed parts had a similar fatty acid composition with around 50% of unsaturated fatty acid, and FAC of all these five parts remained stable during the storage. Other parts of the head, which were not included in the present study, may contain more unsaturated fatty acid as reckoned.

The fins had the highest initial FFA content and a significant increase of FFA contents of all five parts indicated an inevitable quality decrease during the 4-month frozen storage. This suggests the necessity of deactivation of enzymes if the side raw materials need to be stored for long periods even at -25°C

The brain and fins had a high phospholipid content and the significant decrease of PL in the fins during these four months could be explained by the enzyme destruction.

Regarding the lipid oxidation as assessed by PV and TBARS, the gills had both strong primary and secondary oxidation; the primary and secondary oxidation of the eyes increased between 2-month storage and 4-month storage; the fins were very prone to be oxidized and the major primary oxidation was even finished before the samples reached the lab.

Collagen extracted from the raw whole head had a yield of 6.4% and the rest material was also used to a large extent. However, the procedure for fat removing needs to be further optimized.

The results above indicate the values of salmon head and potential usage. The gills would be perfect for general compounds extraction and utilization; the easy-removable fins could be good protein sources; the tongue and eyes would be interesting for lipid extraction; different types of minerals could be derived from gills; and the whole head could be extracted for collagen.

6 Future perspectives

It would be interesting to look deeper into the fatty acids and protein composition of the other rest material from salmon head which were not included in the current project. These parts made up almost 72% of the whole head and might thus also include some valuable compounds for utilization.

It would be worthy if other by-products like skin and backbones could be added, so that the data from head will be comparable within the same species.

The collagen extraction methodology could be further optimized for the salmon heads and the yield might be higher if the whole head could be separated into different parts with the concern of its lipid distribution.

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Appendix A

This appendix Figure 22~~Figure 22~~ shows the temperature change during the 4-month storage. Three temperature loggers were put in the bottom corner, middle of box and outside of the box individually on October 17th, 2018, and they detected the temperature every 66 minutes synchronously. The graph below was based on the data only until February 26th,2019, but the full dataset was collected until April 30th, 2019.

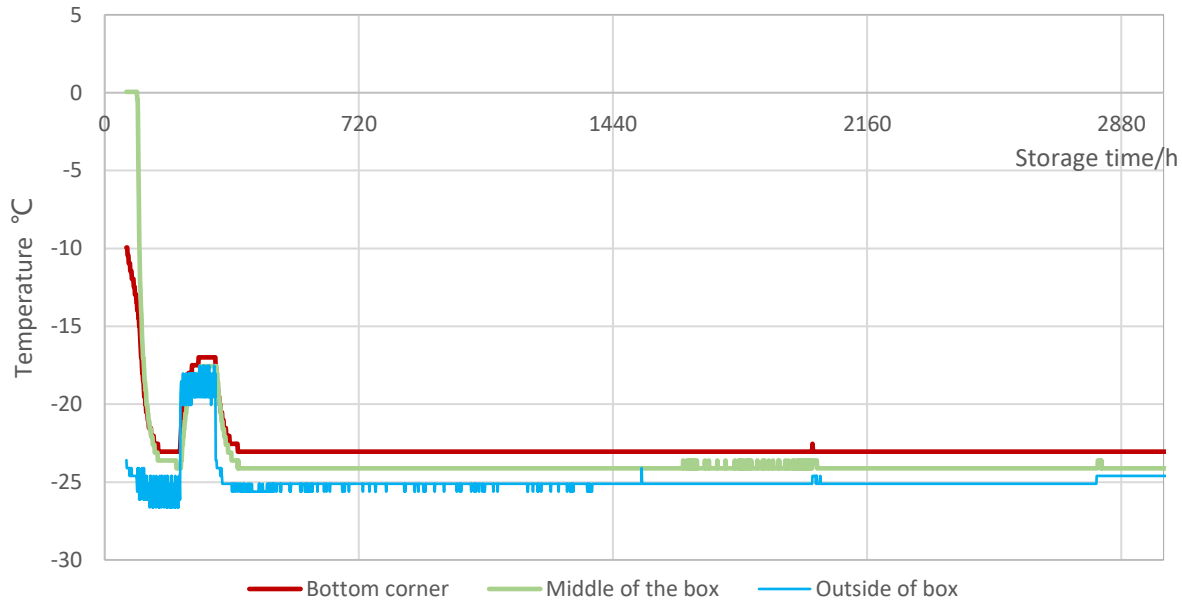

Figure 22 Temperature changes of samples in the freezer during 4-month (2880 hours) storage

Appendix B

This appendix contains tables with values regarding to saturated fatty acid content, monounsaturated fatty acid content, polyunsaturated fatty acid and the ratio of EPA and DHA of five different parts from salmon head during 2-month storage. Independent t-test was done between different parts and paired t-test was done between two batches. Figure 15~~Figure 15~~ was generated based on these tables.

Table 3 Fatty acid composition (g fatty acid / 100g lipids) of the five parts from salmon head when fresh and 2-month old

Material	Components	Fresh	2-month
Brain	SFA	41.7 ± 0.2^{Aw}	41.5 ± 0.5^{Awy}
	MUFA	20.3 ± 0.1^{Aw}	20.2 ± 0.1^{Awy}
	PUFA	34.9 ± 0.4^{Aw}	34.6 ± 0.2^{Aw}
	EPA/DHA	1.1 ± 0.0^{Aw}	1.2 ± 0.0^{Aw}
Tongue	SFA	42.1 ± 0.7^{Aw}	41.2 ± 0.4^{Bwz}
	MUFA	20.1 ± 0.1^{Az}	20.2 ± 0.2^{Awz}
	PUFA	34.9 ± 0.6^{Aw}	35.2 ± 0.2^{Ax}
	EPA/DHA	1.1 ± 0.0^{Aw}	1.2 ± 0.0^{Bw}
Eyes	SFA	42.0 ± 1.2^{Aw}	41.2 ± 0.3^{Bwx}
	MUFA	20.2 ± 0.1^{Aw}	20.3 ± 0.1^{Awx}
	PUFA	35.1 ± 0.4^{Aw}	35.0 ± 0.3^{Awx}
	EPA/DHA	1.2 ± 0.0^{Ax}	1.3 ± 0.1^{Ax}
Fins	SFA	40.5 ± 0.4^{Ax}	40.5 ± 0.5^{Axz}
	MUFA	19.8 ± 0.1^{Ax}	20.0 ± 0.1^{Byz}
	PUFA	36.1 ± 0.4^{Ax}	36.2 ± 0.4^{Ay}
	EPA/DHA	0.8 ± 0.0^{Ay}	0.8 ± 0.0^{Ay}
Gills	SFA	41.8 ± 1.0^{Aw}	42.0 ± 0.4^{Ay}
	MUFA	20.4 ± 0.1^{Ay}	20.5 ± 0.1^{Bx}
	PUFA	34.9 ± 0.3^{Aw}	34.8 ± 0.3^{Awx}
	EPA/DHA	1.0 ± 0.0^{Az}	1.0 ± 0.0^{Az}

^{AB} Different superscript letters at each row indicate significant differences ($p < 0.05$)

^{wxyzv} Different superscript letters at each column indicate significant differences ($p < 0.05$)

Appendix C

This appendix contains tables with individual values regarding to fatty acid composition from five analyzed parts of salmon heads between fresh and 2-month batch.

Table 4 Fatty acid composition (g fatty acid / 100g lipids) of brain when samples were fresh and 2-month old

Fatty acid	Fresh	2 months
C14:0	3.85±0.07	3.91±0.10
C16:0	11.60±0.18	11.58±0.17
C16:1n7	4.44±0.08	4.41±0.04
C17:1	3.12±0.05	3.15±0.18
C18:0	24.85±0.08	24.64±0.22
C18:1n9	3.22±0.01	3.17±0.03
C18:1n7	6.41±0.06	6.42±0.17
C18:2n6	2.32±0.02	2.30±0.07
C18:3n6	0.75±0.01	0.77±0.02
C18:4n3	9.68±0.13	9.71±0.09
C20:0	0.38±0.00	0.40±0.01
C20:1n11	0.74±0.02	0.71±0.01
C20:1n9	0.31±0.01	0.29±0.01
C20:3n6	0.30±0.01	0.28±0.01
C21:0	1.01±0.01	0.97±0.02
C20:3n3	4.24±0.08	4.18±0.06
C20:5n3(EPA)	8.12±0.15	8.16±0.14
C22:1n11	0.84±0.02	0.85±0.01
C22:1n9	0.26±0.00	0.25±0.01
C22:5n3	2.36±0.03	2.23±0.06
C22:6n3(DHA)	7.11±0.06	7.01±0.07
C24:1n9	0.91±0.02	0.97±0.06

Table 5 Fatty acid composition (g fatty acid / 100g lipids) of tongue when samples were fresh and 2-month old

Fatty acid	Fresh	2 months
C14:0	3.99±0.02	3.93±0.07
C16:0	12.52±0.01	12.17±0.14
C16:1n7	4.51±0.01	4.43±0.03
C17:1	3.12±0.06	3.35±0.30
C18:0 0.73	24.19±.04	23.77±0.37
C18:1n9 0.74	3.26±0.01	3.20±0.02
C18:1n7	6.38±0.01	6.42±0.07
C18:2n6	2.34±0.00	2.34±0.03
C18:3n6	0.73±0.00	0.76±0.01
C18:4n3	9.81±0.04	9.97±0.12
C20:0	0.40±0.02	0.40±0.01
C20:1n11	0.75±0.01	0.73±0.01
C20:1n9	0.31±0.00	0.29±0.01
C20:3n6	0.30±0.00	0.29±0.01
C21:0	0.99±0.01	0.98±0.03
C20:3n3	4.28±0.01	4.29±0.02
C20:5n3(EPA)	8.05±0.07	8.28±0.04
C22:1n11	0.86±0.02	0.88±0.02
C22:1n9	0.26±0.00	0.25±0.00
C22:5n3	2.28±0.03	2.21±0.03
C22:6n3(DHA)	7.10±0.06	7.05±0.11
C24:1n9	0.63±0.01	0.66±0.02

Table 6 Fatty acid composition (g fatty acid / 100g lipids) of eyes when samples were fresh and 2-month old

Fatty acid	Fresh	2 months
C14:0	4.03±0.02	3.94±0.16
C16:0	11.38±0.17	11.25±0.18
C16:1n7	4.63±0.09	4.52±0.14
C17:1	2.82±0.04	3.13±0.20
C18:0	25.12±0.25	24.62±0.13
C18:1n9	3.27±0.03	3.20±0.03
C18:1n7	6.76±0.04	6.70±0.09
C18:2n6	2.43±0.01	2.38±0.05
C18:3n6	0.76±0.01	0.77±0.04
C18:4n3	10.08±0.12	10.18±0.24
C20:0	0.39±0.02	0.39±0.01
C20:1n11	0.77±0.01	0.74±0.02
C20:1n9	0.27±0.00	0.26±0.01
C20:3n6	0.30±0.01	0.28±0.01
C21:0	1.05±0.02	1.01±0.01
C20:3n3	4.15±0.08	4.13±0.05
C20:5n3(EPA)	8.29±0.15	8.37±0.22
C22:1n11	0.84±0.01	0.86±0.02
C22:1n9	0.27±0.00	0.26±0.00
C22:5n3	2.34±0.02	2.25±0.03
C22:6n3(DHA)	6.74±0.08	6.65±0.12
C24:1n9	0.62±0.02	0.64±0.04

Table 7 Fatty acid composition (g fatty acid / 100g lipids) of fins when samples were fresh and 2-month old

Fatty acid	Fresh	2 months
C14:0	3.57±0.07	3.680.10
C16:0	12.43±0.11	12.46±0.29
C16:1n7	4.14±0.07	4.19±0.08
C17:1	3.25±0.12	3.37±0.03
C18:0	23.19±0.14	23.08±0.20
C18:1n9	3.11±0.00	3.10±0.06
C18:1n7	6.25±0.04	6.29±0.04
C18:2n6	2.27±0.03	2.23±0.01
C18:3n6	0.70±0.01	0.71±0.01
C18:4n3	9.24±0.07	9.42±0.13
C20:0	0.39±0.02	0.38±0.00
C20:1n11	0.72±0.02	0.71±0.01
C20:1n9	0.58±0.06	0.56±0.03
C20:3n6	0.29±0.00	0.27±0.01
C21:0	0.95±0.01	0.94±0.03
C20:3n3	4.70±0.10	4.67±0.15
C20:5n3(EPA)	7.26±0.11	7.45±0.21
C22:1n11	0.76±0.01	0.81±0.02
C22:1n9	0.24±0.01	0.24±0.00
C22:5n3	2.48±0.08	2.41±0.12
C22:6n3(DHA)	9.14±0.32	9.07±0.32
C24:1n9	0.78±0.02	0.76±0.04

Table 8 Fatty acid composition (g fatty acid / 100g lipids) of gills when samples were fresh and 2-month old

Fatty acid	Fresh	2 months
C14:0	3.96±0.02	4.08±0.12
C16:0	12.47±0.16	12.66±0.15
C16:1n7	4.49±0.06	4.51±0.07
C17:1	3.14±0.06	3.24±0.08
C18:0	24.04±0.16	23.91±0.23
C18:1n9	3.24±0.01	3.22±0.04
C18:1n7	6.64±0.07	6.61±0.10
C18:2n6	2.42±0.03	2.36±0.04
C18:3n6	0.77±0.02	0.78±0.01
C18:4n3	9.62±0.08	9.71±0.06
C20:0	0.38±0.01	0.39±0.01
C20:1n11	0.73±0.01	0.71±0.01
C20:1n9	0.47±0.03	0.49±0.03
C20:3n6	0.30±0.00	0.27±0.01
C21:0	0.98±0.03	0.96±0.02
C20:3n3	4.23±0.05	4.20±0.00
C20:5n3(EPA)	7.75±0.10	7.85±0.10
C22:1n11	0.80±0.03	0.83±0.02
C22:1n9	0.25±0.00	0.24±0.00
C22:5n3	2.26±0.02	2.17±0.02
C22:6n3(DHA)	7.58±0.05	7.51±0.23
C24:1n9	0.64±0.01	0.67±0.03