Master's thesis

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Chemical Composition and Stability of Co-products Obtained from Primary Processing of Farmed Atlantic Salmon (*Salmo salar* L.)

Master's thesis in Biotechnology
Supervisor: Jørgen Lerfall
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Preface

This master thesis was conducted at the Department of Biotechnology and Food Science, Faculty of Natural Science, at the Norwegian University of Science and Technology (NTNU). It marks the completion of a Master of Science in Biotechnology degree. The work on this thesis was carried out from spring 2020 to spring 2021.

I would like to thank my supervisor, Associate Professor Jørgen Lerfall, for guidance throughout the project and especially for helpful feedback on the many drafts of this thesis. In particular, I would like to thank Professor Turid Rustad for fruitful discussions and guidance concerning experimental methodology and result interpretation. I would also like to thank the staff engineer Anne Kathrine Streitlien for help and training during the Kjeldahl protein determination.

Finally, I would like to express my sincere gratitude to my girlfriend, family and friends for their constant love and support during the work on this thesis and throughout my five years of study at NTNU.

Trondheim, May 2021

Didrik Ulleberg

Abstract

The increasing demand for food combined with the need to reduce humankind's environmental footprint makes it crucial to use all available raw materials. The Norwegian aquaculture industry produces large quantities of co-products from Atlantic salmon (*Salmo salar* L.) that are underutilised when used as animal feed. Therefore, the present thesis aims to contribute to the growing knowledge of salmon co-products as ingredients in foods by studying the chemical composition and stability of the residual raw material. The chemical composition was studied by determining the co-products proximate composition, the amount of water- and salt-soluble protein and distribution of fatty acids. Meanwhile, the chemical stability was studied through analysis of the general proteolytic activity, acid value and ATP degradation. The co-products within the scope of this thesis are the belly flap, deboned meat, and Bits & Pieces from farmed Atlantic salmon of Superior and Production quality. The results obtained in the present thesis demonstrate that belly flaps have a higher lipid content and a lower protein content than the filet, while the opposite was true for the manually deboned meat fraction. The Bits & Pieces showed a varying proximate composition depending on how it was produced. All investigated co-products had a similar distribution of specific fatty acids as the filet. Furthermore, the fatty belly flap and Bits & Pieces were more susceptible to ATP degradation than the leaner deboned meat and filet. The findings also suggest that the belly flap might have increased levels of general proteolytic activity. Importantly, it was demonstrated that co-products from Superior and Production quality salmon have similar chemical composition and stability. The preliminary results suggest that manually produced co-products from both Superior and Production quality salmon contain valuable nutrients suited as ingredients in food production. Moreover, the results indicate different functional properties of the fractions that must be accounted for, such as the increased fat content in belly flaps. Further efforts must be made to produce salmon co-products of consistent and high quality at an industrial scale and to develop food products accepted by the market.

Sammendrag

Menneskehetens behov for mat øker samtidig som vi må redusere vårt økologiske fotavtrykk, noe som gjør det viktig å utnytte alle tilgjengelige ressurser. Norsk akvakultur produserer i dag store mengder koprodukter fra Atlantisk laks (*Salmo Salar* L.) som ikke blir optimalt utnyttet når det blir brukt som dyrefor. Derfor er denne avhandlingens mål å bidra til den økene kunnskapen om koprodukter fra laks som en ingrediens i mat, ved å kartlegge den kjemiske sammensetningen og stabiliteten til råstoffet. Den kjemiske sammensetningen til koproduktene ble studert ved en proksimalt komposisjonsanalyse, mengden vann- og salt-løselige proteiner og fettsyredistribusjonen. Den kjemiske stabiliteten ble undersøkt ved analyse av generell proteaseaktivitet, syretall og ATP nedbrytning. Koproduktene som studeres er buklist, skrapekjøtt og avskjær fra Atlantisk oppdrettslaks av både Superior- og Produksjonskvalitet. Resultatene oppnådd i denne avhandlingen viser hvordan buklisten har et høyere fettinnhold og lavere proteininnhold enn fileten, mens det motsatte gjelder for manuelt produsert skrapekjøtt. Avskjæret har en varierende sammensetning avhengig av hvordan det er produsert. Fordelingen av spesifikke fettsyrer var lik i alle koproduktene og i fileten. Det demonstreres at ATP raskere brytes ned i koprodukter som er rike på fett, som buklist og avskjær, sammenliknet med det magrere skrapekjøttet og fileten. Buklisten viser også tegn på å ha høyre nivåer av generell proteaseaktivitet sammenliknet med de andre fraksjonene. Et viktig funn er at koprodukter fra Produksjonslaks har like god kjemisk komposisjon og stabilitet som koprodukter fra Superiorlaks. Undersøkelsene gjort i løpet av dette prosjektet viser at manuelt produserte koprodukter fra Superior- og Produksjonskvalitet laks inneholder verdifulle næringsstoffer velegnet til matproduksjon. Resultatene viser også hvordan de forskjellige koproduktene har ulike funksjonelle egenskaper som det må bli tatt hensyn til, slik som det økte fettinnholdet i buklist. Videre arbeid må bli gjort for å produsere koprodukter fra laks av en jevn og høy kvalitet i industriell skala, og for å utvikle produkter akseptert av markedet.

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Abbreviations and Nomenclature

NTNU	Norwegian University of Science and Technology
ISO	International Organization for Standardization
FAO	Food and Agriculture Organization of the United Nations
UNDP	United Nations Development Program
Sup	Superior
Prod	Production
CFU/g	Colony forming units per gram
WSP	Water-soluble protein
SSP	Salt-soluble protein
GPA	General proteolytic activity
GC	Gas Chromatography
FA	Fatty acid
FFA	Free Fatty Acids
SAF	Saturated fatty acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
HPLC	High Performance Liquid Chromatography
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
IMP	Inosine monophosphate
Ino	Inosine
Hx	Hypoxanthine
h	Hours
min	Minutes
s	Seconds

1. Introduction

1.1. Scopes and Research Objectives

The aim of this MSc project is to study the chemical composition and stability of co-products from farmed Atlantic salmon (*Salmo salar* L.). Furthermore, the thesis aims to investigate the stability and composition of residual raw material from Production quality salmon compared to Superior quality salmon. This work will contribute to the growing knowledge of salmon rest raw materials as ingredients in food production. The project's long-term goal is to increase the sustainability, and potentially the profits, of salmon aquaculture by using more co-products directly for human consumption.

The co-products within the scope of this thesis are the deboned meat from frames, belly flaps, and Bits & Pieces. These co-products were chosen since they contain most of the meat left after filleting that could be used to produce salmon mince products intended for human consumption. Salmon filets will be studied in parallel with the co-products and used as a reference point. Finally, raw material from both Superior and Production quality salmon will be analysed and compared.

The composition of the salmon raw material will be studied through a proximate analysis, which includes the percentage content of water, protein, lipids, and ash. Furthermore, the content of water- and salt-soluble protein and the fatty acid profile will be analysed. The chemical stability of the raw material will be assessed through analysis of the general proteolytic activity, acid value, and ATP degradation. These parameters will be measured both in salmon samples frozen down directly after processing and on salmon samples stored in a refrigerated room on ice (0-4 °C) for seven days.

1.2. Previous Studies

This section will present three previous studies aiming to increase the amount of salmon co-products used for human consumption. They provide a foundation for the work continued in this thesis.

1. “Utnyttelse av kjøtt fra ryggbein av laksefarse og skrapekjøtt“ by Østvik et al. (2005)
2. “Kommersielle produkter av ryggbeinskjøtt fra laks“ by Østvik & Grimsmo (2010)
3. “Restråstoff fra atlantisk laks (*Salmo salar* L.). Kartlegging av mikrobiota og stabilisering av råstoff“ by Sletten (2020)

By the report “Utnyttelse av kjøtt fra ryggbein av laksefarse og skrapekjøtt“ conducted by SINTEF Ocean, Østvik et al. (2005) aimed to establish an industry standard for the production of deboned meat from salmon frames. Their main finding was that mechanically deboned meat from salmon could be produced in a high quality suited for food production. Furthermore, they found that the composition and quality of deboned meat vary with the amount of meat removed from the backbone. The quality decreases while the fat content increases when too much meat is removed from the frames due to the inclusion of soft, fatty tissue. The fat content in the deboned meat varied from 6-23%. The deboned meat had acceptable microbial levels and autooxidation rates. Finally, they concluded that the industry show interest in the production and product development of deboned meat.

Østvik & Grimsmo (2010) continued their work with the SINTEF Ocean report “Kommersielle produkter av ryggbeinskjøtt fra laks“. Here, they aimed to produce deboned meat from salmon frames at an industrial scale, use it for product development of 3-5 new mince products, and evaluate how the market received the products. The quality of the mechanically deboned meat varied, where some batches were of suitable quality while other was of unacceptable quality regarding taste, colour, and consistency. The low-quality deboned meat had a fat content of 21.9% and contained 57% red meat and 43% soft tissue. Because of the varying quality of the deboned meat, they did not produce new mince products that could be evaluated by the market. Østvik & Grimsmo concluded that the industrial-scale production of mechanically deboned meat from salmon frames requires further optimization before it can be produced with consistent and high quality.

The varying quality of deboned meat combined with low interest from the industry led Østvik & Grimsmo (2010) to conclude that the commercial potential of deboned meat in Norway was low. SINTEF Ocean has not done any further studies on the topic (Grimsmo, personal communication, May 5, 2021). However, the awareness of sustainability and food waste reduction has increased since 2010 (FAO, 2019). This could renew the industries, food producers, and markets interested in utilizing salmon co-products for human consumption.

The present thesis is a direct continuation of the work done by Sletten (2020) in her master thesis “Restråstoff fra atlantisk laks (*Salmo salar* L.). Kartlegging av mikrobiota og stabilisering av råstoff“. Sletten aimed to assess the microbial stability of salmon rest raw materials concerning their suitability for human consumption. In similarity to Østvik & Grimsmo (2010), she found that the quality of salmon co-products varied depending on production method and site. Sletten finishes her thesis by recommending further studies to assess the chemical composition and stability of salmon rest raw material, which will be done in this thesis.

2. Theory

Insufficient utilisation of food resources remains a significant problem regarding the growing world population, the increased demand for food, and the urgent need to reduce our environmental impact. In 2019, FAO estimated that 14% of the global food produced for human consumption is lost between harvest and the retail level (FAO, 2019). The United Nations 12th Sustainable Development Goal calls for a more responsible and sustainable utilisation these food resources (UNDP, 2017).

Article 4 of the revised EU Waste Framework Directive (2008) introduces a food waste hierarchy that differentiates between utilisation grades of food waste (EU, 2008). For example, the framework categorizes food waste used as animal feed as a more environmental and economical utilisation than landfill. Stevens et al. (2018) categorize the utilisation grade of fish rest raw material in their adaption of the US Environmental Protection Agency's *Food Recovery Hierarchy* (EPA, 2021) (Figure 2.1). The hierarchy categories fish rest raw material used directly for human consumption as the highest value and most preferred option. The raw material loses value if it is downgraded to animal feed, fertilizer, or biofuel. The least preferred option is to dispose of the raw material as landfill.

Most of the rest raw material produced by Norwegian aquaculture today is used as animal feed, while only a tiny fraction are used as human food (Myhre et al., 2020). The aquaculture industry could increase food production, increase their economic growth, and reduce their environmental footprint by using more of the fish directly for human consumption (Asche et al., 2018; Stevens et al., 2018). However, the commercialization of foods based on salmon co-products is a difficult process. It requires the industrial-scale production of co-products with high and stable quality, development of products with consumer acceptance, and a marked willing to buy the products (Olsen & Tobiassen, 2004; Østvik & Grimsmo, 2010).

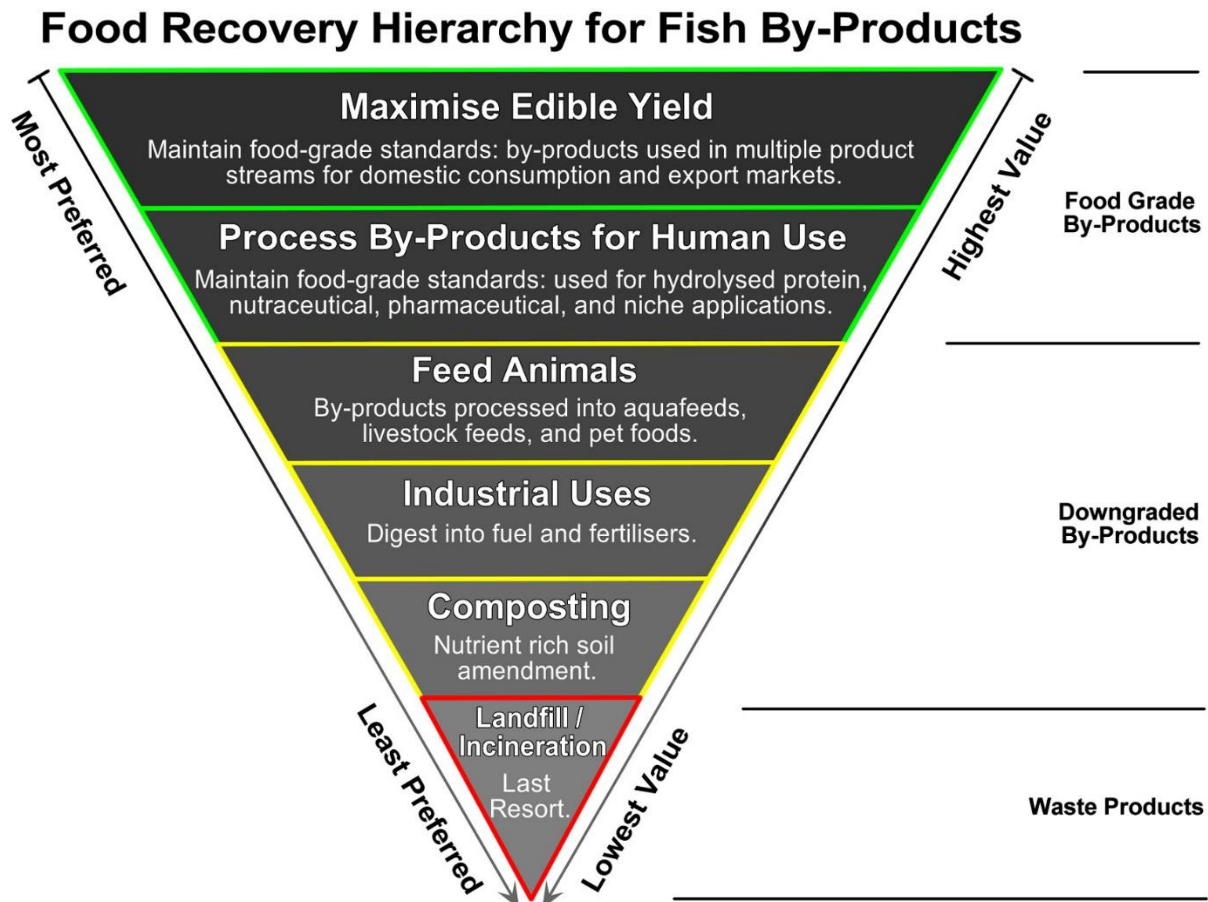


Figure 2.1. Food Recovery Hierarchy for fish residual raw material. Rest raw material utilised for human consumption are rated as more valuable than rest raw material used for animal feed or landfill. Stevens *et al*. uses the term By-Products instead of residual raw material. Adapted from "The rise of aquaculture by-products: Increasing food production, value, and sustainability through strategic utilisation" by Stevens et al. (2018).

2.1. Residual Raw Materials

2.1.1. Definition of Co-products and By-products

The terms residual raw materials, co-products, and by-products are given different definitions in the literature. This thesis will use the definitions used by Aspevik et al. (2017), which is based on the definition set by the European parliament (Figure 2.2). In meat and fish processing, the main products are what the industry wants to produce and sell, such as filets, chops, and mince. Residual raw material, or rest raw material, is defined as everything that is not the main product. Examples of residual raw material are skin, viscera, heads, hooves, and trimmings. The residual raw material is divided into co-products and by-products. Co-products are rest raw material suitable for human consumption, while the by-products are unsuitable for human consumption. Finally, the by-products are divided into three categories, where only category three is suited to be used as animal feed.

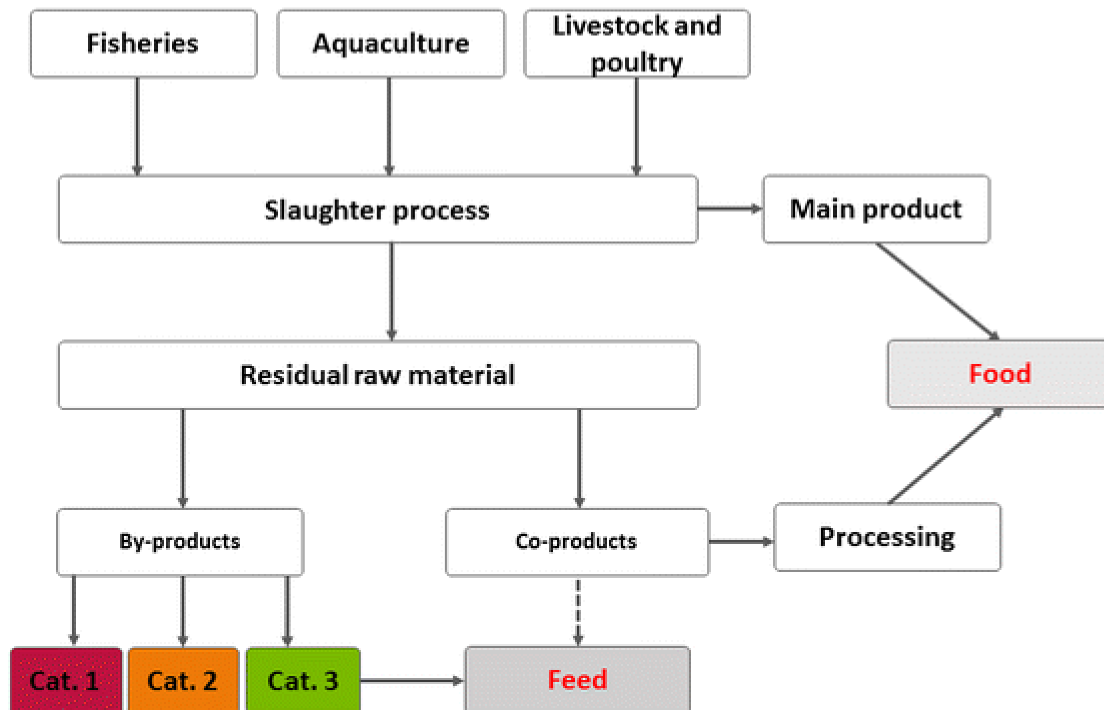


Figure 2.2. Classification scheme of raw materials during the processing of fish, livestock, and poultry. Adapted from "Valorisation of Proteins from Co- and By-Products from the Fish and Meat Industry" by Aspevik et al. (2017).

2.1.2. Distribution of Residual Raw Materials in Salmon

Olsen & Tobiassen (2004) reported that the filleting yield of Atlantic salmon varies from 45% to 62% of total wet weight depending on trimming grade. The remaining 38-55% are categorized as residual raw material. Figure 2.3 illustrates the distribution of residual raw material in salmon when 41.5% of the wet weight is regarded as rest raw material. The trimmings weight of 2% and the belly flap weight of 1.5% vary with filleting grade.

In percentage of total wet weight, trimmed salmon frames consist of approximately 36% red meat, 14% soft fatty tissue, and 50% bone (Olsen & Tobiassen, 2004; Østvik et al., 2005). The deboned meat co-product fraction is produced from the meat on the frames. Meanwhile, the belly flap co-product fraction consists of meat and fat on the belly flaps. Finally, The Bits & Pieces co-product fraction can consist of both trimmings and of the filet's anterior and posterior cuts.

The salmon head constitutes 10% of the salmon's wet weight and contains meat that could be removed industrially. The potentially high-quality meat makes salmon heads a fourth co-product of interest for this study. However, they are excluded from the scope of this thesis due to the low yield and varying quality of industrially produced meat from salmon heads.

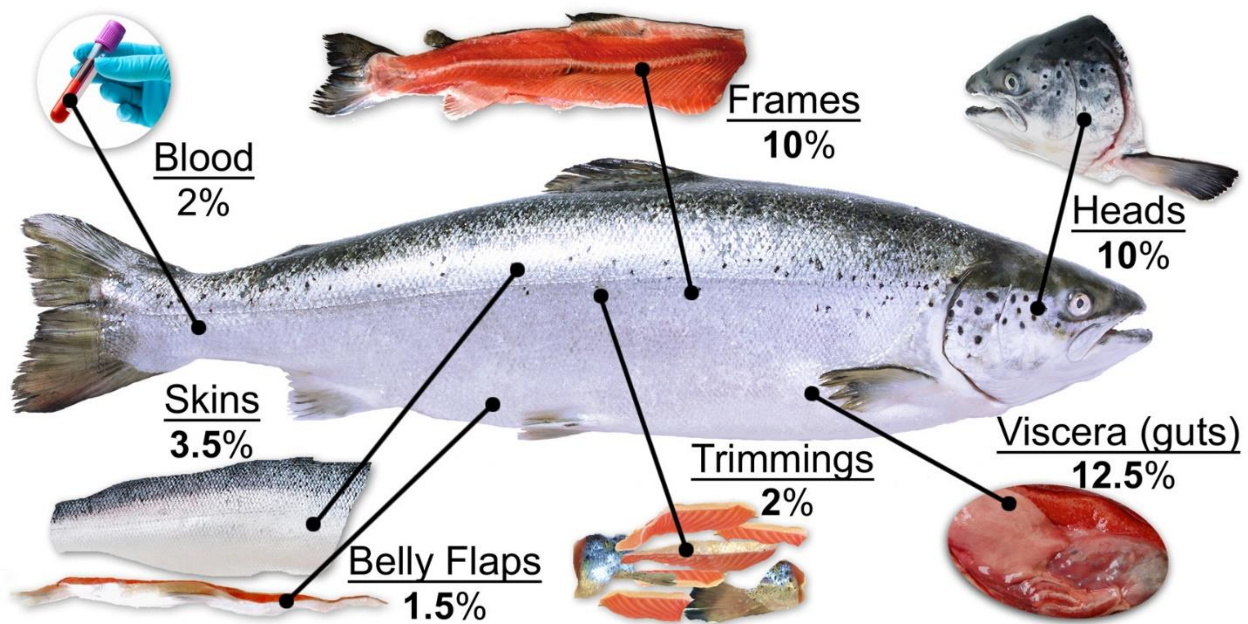


Figure 2.3. Distribution of residual raw material from Atlantic Salmon as percentage of total wet weight. The weight of the trimmings and belly flaps can be higher than the presented values, depending on how the fillet are cut. Adapted from "The rise of aquaculture by-products: Increasing food production, value, and sustainability through strategic utilisation", by Stevens et al. (2018).

2.2. Utilisation of Salmon Co-products

2.2.1. History

It is by no means a novel idea to use residual raw materials from fish for human consumption. Nordic countries, like Norway and Island, have long traditions of utilizing such rest raw materials. Like many other countries during the ages, Norway and Island have endured periods of food scarcity, giving an incentive to utilize the available resources. Examples are the traditional drying of cod heads and tongs, the Icelandic fermented shark (Hákarl), and cod liver oil. The first recorded use of cod liver oil dates back to England in 1783 (Curtis et al., 2004).

More effective harvesting, processing, and distribution methods in the later 19th century enabled Nordic countries to produce marine food for export (Hjellnes et al., 2020). At the same time, growth in prosperity allowed consumers to have higher quality requirements. This development caused the focus to shift from maximum utilisation of available resources to the production of high value, easily exported products. Moreover, the consumer preferences of fish have changed from buying whole gutted fish at specialized retailers to buying portion packaged fillets and ready to eat products at supermarkets (Carlucci et al., 2015; Olsen & Tobiassen, 2004).

The sale of fillets and ready-to-eat products instead of whole fish introduces a significant change: the rest raw materials are now generated in the industry's processing facilities instead of the consumers' homes. For that reason, the responsibility of utilizing residual raw materials sustainably is in the industry's hands. The growing awareness of sustainable production makes this responsibility more important than ever.

2.2.2. Today's Situation

Norwegian aquaculture produced 1.452.928 tons of live weight salmon and trout in 2019 (SSB, 2021). The production resulted in 458.200 tons of available rest raw material (Myhre et al., 2020). Some of the rest raw material becomes unavailable for the Norwegian industry when salmon is exported as gutted whole fish for further processing in other countries. Norway exported 1.179.634 tons of salmon and trout in 2019 (Norges Sjømatråd, 2021).

Myhre et al. (2020) estimate that 93% of the rest raw material from aquaculture available for the Norwegian industry were utilised in 2019. The remaining 7% unused material is mostly blood that is challenging to collect. 75-80% of the available rest raw material are used as components in animal- and fish feed through ensilage or the production of oil and protein isolates. 10-15% are used for biogas production, while only 2% of the available rest raw material are used to produce products intended for human consumption. The co-products used for human food are mainly mince-based products containing meat from trimmings, belly flaps, or heads.

In contrast with the Norwegian aquaculture industry, the Scottish industry in 2015 used 15% of their residual raw material directly for human consumption, 75% for animal feed, and 10% for fuel or fertilizer production (Stevens et al., 2018). Figure 2.4 shows a schematic overview of this distribution, as well as the value of the materials. Stevens *et al*. argued that the revenue from aquaculture rest raw materials could be increased by 803% in a theoretical scenario where most edible co-products are used for human consumption. This highlights the potential economic growth the Norwegian industry could gain by utilizing more salmon co-products for this purpose.

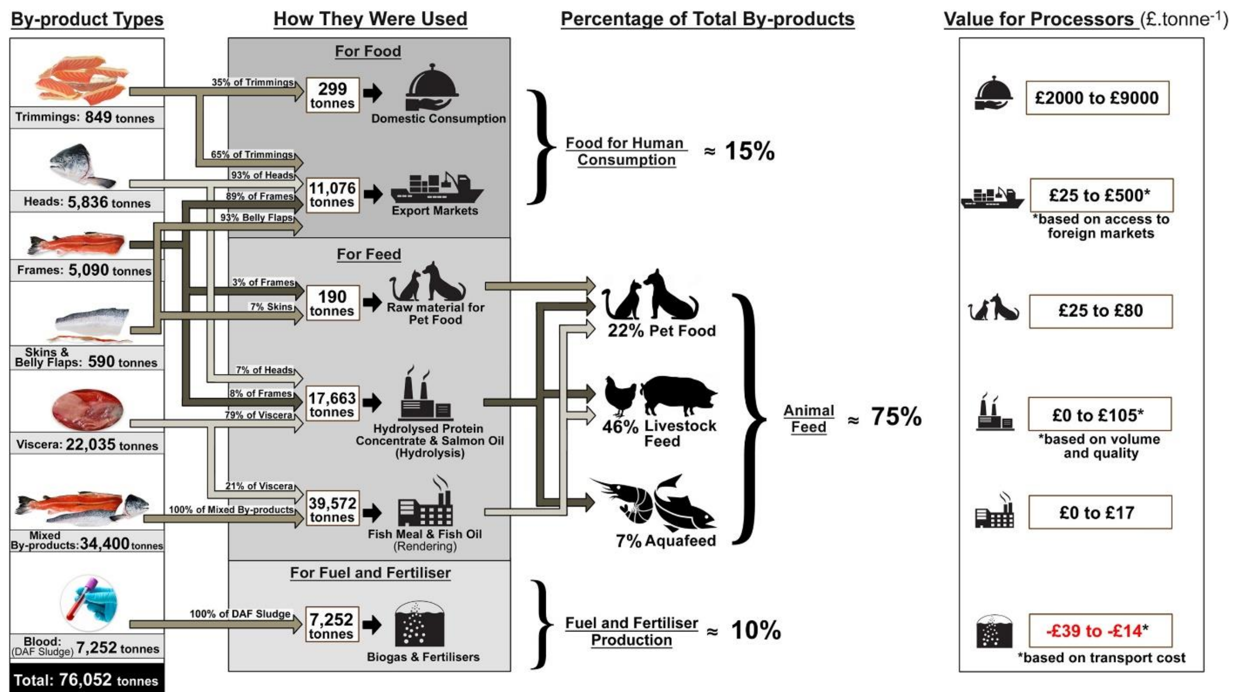


Figure 2.4. Utilisation of residual raw material in the Scottish aquaculture industry, 2015. 75% of the residual raw material are used as animal feed, while 15% are used for human consumption. Most of the material used for human consumption are exported the Asian and African markets. Sevens *et al*. uses the term by- products instead of residual raw material. Adapted from "The rise of aquaculture by-products: Increasing food production, value, and sustainability through strategic utilization" by Stevens et al. (2018).

2.2.3. Examples of Food Products Based on Salmon Co-products

The significant amounts of high-quality raw material available, combined with increasing consumer demands for ready-to-eat products, has led the industry to develop products that use salmon co-products as ingredients. Mince-based products are well suited for this purpose as the mince can be produced from meat left on frames, belly flaps, and trimmings. Many products combine lean white fish with fattier salmonids, or even bovine fat, to achieve the desired functional properties. Producers also experiment with spices and smoking to achieve good taste.

Nofima performed two surveys in 2001, one at the "European Seafood Exposition" in Brussel and one at "ANUGA" in Cologne (Olsen & Tobiassen, 2004). Their goal was to identify existing products on the market that are, or could be, based on fish co-products. One of their findings was that some salmon slaughterhouses produce two types of blocks of frozen co-products exported for further processing. One block consists of chunky pieces of trimmings from filleting. The other type consists of mince produced from frames and smaller trimmings. Furthermore, they found a series of mince-based products in the form of sausages, patties, and breaded salmon. These findings suggest an interest in the market of 2001 to use salmon rest raw materials for human consumption.

Several food products that contain salmon co-products are today available in Norwegian supermarkets. One example is “Mils Laksepålegg” which is a spread containing 40% hot smoked salmon, where the salmon are trimmings left after the production of smoked salmon fillets (Figure 2.5, left) (Mills forbrukerservice, personal communication, March 10, 2021). The spread comes with two seasonings, one with spring onion and lemon, and one with pepper. Another example is the “Salma Burger”, a paté made from chunky salmon pieces where filet trimmings could be a suited ingredient (Figure 2.5, right). These burgers are seasoned with pepper and lime, fresh herbs, or sold without seasoning (not included in the picture).

Figure 2.5. Examples of products that are, or could be, based on salmon co- products available in supermarkets in Trondheim, Norway, 2021. *Left,* “Mills Laksepålegg” with two different seasonings. *Right,* Salma Burgers with two different seasonings.

2.2.4. Sustainable Utilisation of Salmon Co-products

As previously illustrated in Figure 2.2, the edible parts of the rest raw materials, the co-products, can either be used for human consumption or animal feed. The salmon co-products intended for human consumption are used as ingredients for foods and eaten directly by humans. The rest of the co-products are mainly used to produce ensilage that is fed to livestock or fish that humans subsequently eat. Thus, both the co-products used directly in mince products and the parts used for ensilage production ends up as human food in the end. The critical difference is that the second alternative introduces one extra trophic level in the food chain affecting the sustainability of its utilisation. Only 5-20% of the energy of one organism is retained in the next organism in a food chain (Cottrell et al., 2021). The inefficient energy transfer means that 80-95% of the energy is expended between each trophic level of a food chain. The energy is expended by activities such as movement, heat production, and growth. This fundamental law of nature also applies to the two possible applications of salmon co-products. The most energy-efficient, and thereby the most sustainable alternative, are to use the co-products directly for human consumption.

2.2.5. Challenges in Utilising Salmon Co-products for Human Consumption

We have now established that it is sustainable and potentially economically feasible to utilize salmon co-products for human consumption. Since this is the case, why do not Norwegian producers utilize more than a small fraction of the co-products for this purpose? The following chapter identifies the main obstacles toward better utilisation of the available co-products.

Industrial Production of Salmon Co-products

In Norway, co-products intended for human consumption must be handled according to the Norwegian regulations on the quality of fish and fish products (Lovdata, 2013). This means that the first step towards more sustainable utilisation of co-products starts at the processing facility. The aquaculture industry already has the infrastructure and knowledge necessary to process filets, which is a good foundation. The co-products must be handled with the same care as the fillets to retain their food-grade quality. Correct handling includes a consistent cold chain, clean instruments, and separation from other fractions such as blood and viscera rich in proteolytic enzymes and spoilage bacteria (Falch et al., 2006).

Østvik et al. (2005) and Østvik & Grimsmo (2010) found that the industrial production of high quality deboned meat from salmon was possible but require further optimization. The produced deboned meat was of varying quality depending on the production line and had a relatively short shelf life. One of the difficulties with Bits & Pieces is to remove all bones from the product. These challenges mean that the industrial production of high quality salmon co-products would require research and development (R&D), time and investment costs.

Product Development

When high quality, food-grade co-products are produced, they should be used as ingredients in food intended for human consumption, which requires product development. Østvik & Grimsmo (2010) has previously shown that it is possible to produce salmon burgers based on deboned meat of acceptable quality and taste. These burgers consisted of only deboned meat with little seasoning. In further product development, it would be interesting to experiment with different ingredients and seasonings that could enhance the mince's flavour and stability (Coulate, 2016). Examples of ingredients that could increase the quality of the products are bovine fat, white fish, smoked fish, vegetables, and protein hydrolysates. Moreover, products could consist of a combination of salmon filets and co-products if foods made from only co-products are of lesser quality.

Consumer acceptance

One of the biggest challenges in the utilisation of salmon co-products is consumer acceptance. It does not help that it is possible to produce food from salmon co-products if no one buys them. In a comparative study between the broiler and salmon industries, Asche et al. (2018) pointed to the successes of the broiler industry. The broiler industry introduced several co-product based value-added products to the market, which created a rapid market expansion. Asche *et al.* argued that the salmon industry could experience a similar growth by producing a more diverse range of products.

Carlucci et al. (2015) reported that consumers prefer fish products that appears "natural" with little processing. That consumer preference might be a challenge since most salmon co-products must be produced as mince used in further processing. On the other hand, they also report that consumers prefer eco-labelled seafood products. The consumer acceptance of co-product-based foods could increase by highlighting the sustainable aspect of eating salmon co-products.

Economy

The aquaculture industry follows the market, which means that the salmon co-products will be utilised for human consumption when it is economically favourable. The investment costs of industrial-scale production and product development of food containing co-products need to be balanced by the generated revenue. Østvik et al. (2005) estimated that the production of deboned meat would cost 33 NOK/kg in 2005, and that the burgers made from deboned meat could be sold to supermarkets with an estimated price of 40-45NOK/kg. He summarizes that the successful development of commercialized products based on salmon co-products requires product development in parallel with optimization of the industrial co-product production.

2.3. Quality of Salmon as a Raw Material

2.3.1. Superior and Production Quality

The Norwegian aquaculture industry sort all fish according to their physical quality, as described in the Norwegian industry standard for fish (NISF, 1999). The standard defines three quality gradings: Superior (hereby referred to as Sup), Ordinary, and Production (herby referred to as Prod) quality. The Ordinary quality class is not used by the industry today and are therefore excluded from the study.

Sup quality fish is defined as a product without substantial faults, damages, or defects suitable for all purposes. Fish with substantial damage or defects are sorted as Prod quality fish. Normal faults are sexual matured characteristics, deformities, severe handling defects, or scale loss and internal quality defaults such as melanin colouration in the muscle. Jensen et al., (2011) estimated that approximately 10% of the produced salmon in 2010 were categorized as Prod quality. The portion of Sup and Prod quality salmon vary between different locations and years.

Prod quality salmon can only be sold to approved establishments in Norway that can process the salmon to rectify the defects. These fish are fit for human consumption and contain valuable nutrition, regardless of their deformities (Olsen & Tobiassen, 2004).

2.3.2. Variations in the Proximate Composition of Salmon

The proximate composition of foods is defined as the composition of water, ash, lipid, protein, and carbohydrates presented as a percentage weight of total wet weight ("Proximate Composition Analysis," 2016).

The proximate composition of salmon varies between individual fish. The main factors affecting the composition are endogenous factors such as sex, life cycle stage and size, and exogenous factors such as diet composition, amount of feed, temperature, salinity, and season (Shearer, 1994). Mørkøre & Rørvik (2001) found that the fat content of farmed Atlantic salmon decrease during winter and increase from summer to autumn. Furthermore, Mørkøre et al. (2010) found that the concentrations of Hx are negatively correlated with water temperature, indicating that salmon harvested in the winter might have a shorter shelf life than salmon harvested during summer.

The proximate composition also varies within each fish. Katikou et al. (2001) found the contents of lipids in the fillet to vary between 2% in the posterior edge to 18% in the belly flap area. Furthermore, the content of water and lipids are inversely proportional and constituted 80% of the filets wet weight. The remaining 15-20% of the filet wet weight are protein and ash.

The varying composition of salmon can create challenges in the production of mince products. Mince products are often emulsions or gels, where the water, lipid, and protein content influence the product's functional properties (Coulate, 2016). Producers that wish to produce food products based on frozen blocks of co-products should be aware of the proximate composition and functional properties of the raw material they are using.

2.4. *Post-mortem* Processes in Salmon

The shelf life of food is defined as the finite time from which a food product is produced until it is no longer fit for consumption under specific storage conditions (IFST, 1993). What processes occur inside a salmon that makes it unfit for consumption? This is a highly relevant question for salmon processors, which has been paid much attention. The answer to the question can be divided into three parts: autolysis, microbial activity, and lipid oxidation.

2.4.1. Rigor Mortis and the Phases of Deterioration

Muscles lose their supply of oxygen upon death. These anaerobe conditions start the process of *rigor mortis*, where muscle tissue contracts. The resulting stiff and rigid body causes the name *Rigor mortis*, which translates to “stiff death”. The lack of oxygen causes glycolytic enzymes to convert glucose into lactic acid for ATP production, which decreases the muscle's pH. Acid active enzymes such as cathepsins then activates and starts to degrade muscle proteins. *Rigor mortis* ends when these enzymes have broken down the muscle sufficient enough for them to relax (Huss, 1995; Wang et al., 1998).

The endogenous enzymes continue to break down cellular components weeks after death together with microbial activity and lipid oxidation. Some of these processes increase the quality of the products, giving the muscle a desirable texture, taste, or colour (Huss, 1995). However, the final result of the deterioration processes is a product unfit for human consumption, marking the end of the product's shelf life.

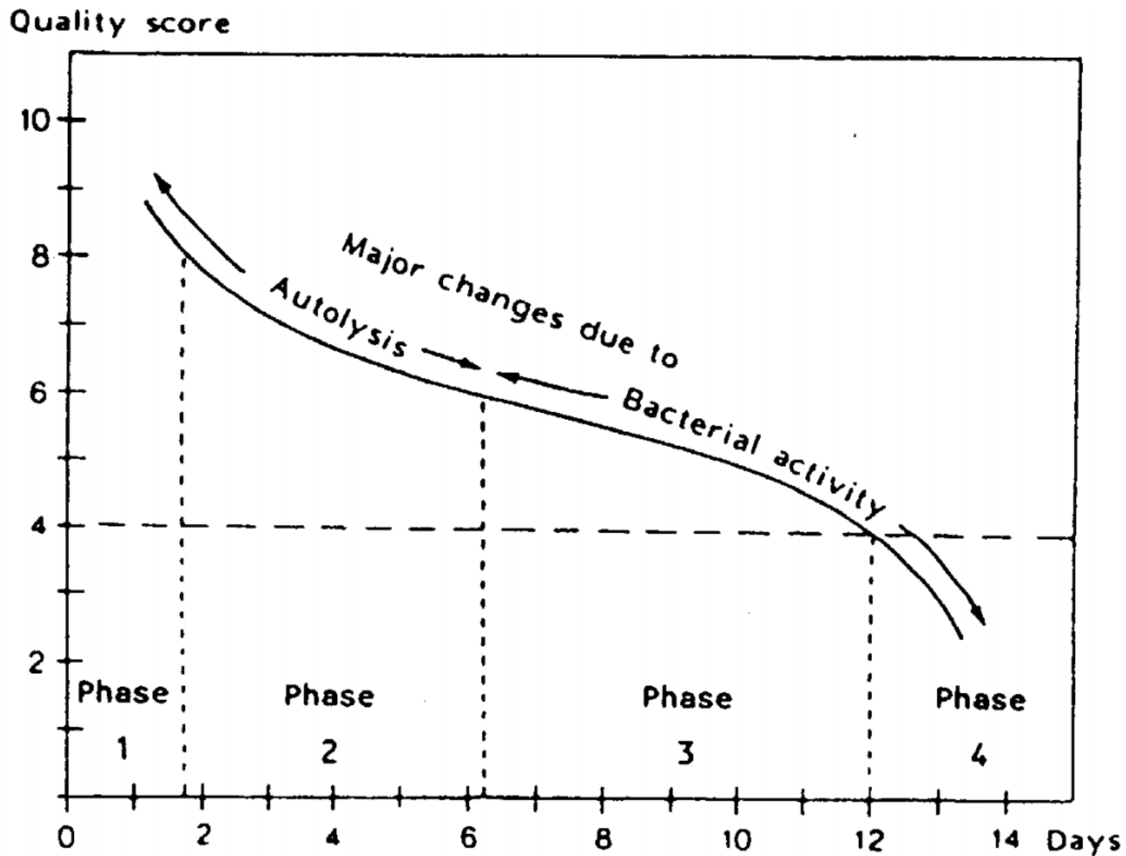


Figure 2.6. Changes in the eating quality during iced (0 °C) storage of cod due to autolysis and bacterial activity. Adapted from "Storage life of gutted and unsoiled white fish" by Huss & Asenjo (1976).

In an article later referred to in "Quality and quality changes in fresh fish" by FAO (Huss, 1995), Huss & Asenjo (1976) defines four phases of deterioration for cod stored at 0 °C (Figure 2.6). The four deterioration phases are similar in salmon. The sensory quality of the cooked cod is assigned a 0-10 score, where 10 are the best score, and 4 is the rejection level. During phase 1 and 2, autolytic enzymes break down compounds associated with the taste of fresh fish and cause a softening of the tissue. The fish enters phase 3 when bacterial growth starts to cause off-flavours. In phase 4, the fish are characterized as spoiled and have reached an end of their shelf life.

2.4.2. Autolysis

Autolysis is the *post-mortem* process where endogenous enzymes break down cellular structures, lipids, proteins, and nucleotides. Autolysis is the primary deteriorating process the first days *post-mortem* before the bacterial activity becomes a problem in phase 3 and 4 (Figure 2.6). The rate of autolysis is affected by the handling and storage conditions of the fish (Ghaly et al., 2010). Both the blood and intestines are rich in autolytic enzymes, so separating these fractions from the meat would extend the shelf life. Furthermore, the activity of endogenous enzymes is slowed by storage at low temperatures (Stoknes & Rustad, 1995).

Autolysis causes deterioration of the raw material both directly and indirectly. First, the activity of endogenous enzymes produce compounds that directly cause undesirable flavours, like hypoxanthine (Hx), bitter peptides, and formaldehyde (Hansen et al., 1996). Secondly, autolysis indirectly causes deterioration by facilitating lipid oxidation and bacterial growth. Lipid oxidation is facilitated when lipases convert relatively stable triglycerides and phospholipids into easily oxidized free fatty acids (FFAs). Bacterial growth is facilitated when autolytic proteases produce free peptides and amino acids which is an excellent growth substrate for bacteria (Huss, 1995).

The ATP level drastically decreases the first 24-hours *post-mortem* due to the lack of oxygen (Wang et al., 1998). ATP (Adenosine triphosphate) are converted to ADP (Adenosine diphosphate) when used for energy, and the ADP is subsequently converted to AMP (Adenosine monophosphate) and IMP (Inosine monophosphate) by autolytic enzymes. IMP is associated with the umami flavours of fish and shellfish associated with a pleasant and slightly sweet taste. However, IMP is further converted to Ino (Inosine) and Hx during phase 2 of deterioration (Figure 2.6). Hx accumulation is associated with a loss of flavour and produces a bitter undesirable taste (Hong et al., 2017).

The ATP degradation during autolysis is used to estimate the freshness of fish through the K-, Ki-, and H-values calculated by Equation I, II and III, respectively (Hong et al., 2017; Karube et al., 1984; Saito et al., 1959).

$$\textit{K-value} = \frac{[(Ino+Hx)]}{[(ATP+ADP+AMP+IMP+Ino+Hx)]} * 100 \quad \text{Equation I}$$

$$\textit{Ki-value} = \frac{[(Ino+Hx)]}{[(IMP+Ino+Hx)]} * 100 \quad \text{Equation II}$$

$$\textit{H-value} = \frac{[(Hx)]}{[(IMP+Ino+Hx)]} * 100 \quad \text{Equation III}$$

The K-value is recognized as one of the most effective indicators of fish freshness (Hong et al., 2017). As shown in Equation I, the K-value is the ratio of ATP-related compounds converted to Ino and Hx. Saito et al. (1959) recommended that fish with a K-value of 20% are rated as very fresh, 50% moderately fresh, and 70% as not fresh. The Ki-value (II) is a simplified version of the K-value that excludes ATP and ADP, and AMP, as the concentration

of these compounds rapidly decreases the first 24 hours *post-mortem* (Karube, 1984). The K- and Ki-values are most accurate as indicators of fish freshness during phase 1 and 2 of the deterioration process (Figure 2.6), as deterioration in later phases mainly are caused by microbial activity rather than autolysis.

The H-value (III) is similar to the K-value but represents the fraction of ATP-related compounds completely broken down to Hx. The H-value is a good indicator of the development of bitter taste caused by Hx. The H-, K- and Ki-values also include Hx produced by spoilage bacteria like *Pseudomonas* spp., *Shewanella putrefaciens,* and *Photobacterium phosphoreum* (Hong et al., 2017).

2.4.3. Microbial Activity

Microorganisms are mainly found on the skin, gills, and intestines of fish when it is alive. The species and density of microorganisms depend on the environment of the fish. The fish muscle is usually sterile as the immune system prevents bacterial growth. The immune system collapses upon death, allowing bacteria to enter the muscle and proliferate (Huss, 1995).

The type of spoilage bacteria active in fish is highly dependent on fish species and the conditions of storage. For example, cod stored at 0 °C in aerobic conditions is typically spoiled by species of gram-negative bacteria such as *Pseudomonas* spp. and *Shewanella* spp. (Ghaly, 2010). Spoilage bacteria produce volatile compounds such as trimethylamine (TMA), sulphides, ketones, aldehydes, and esters that result in an undesirable flavour and texture of the fish muscle. These processes result in sensory rejection when the microbial levels reach 10^6-10^9 CFU/g (Hansen et al., 1996; Kuuliala et al., 2018; Mikš-Krajnik et al., 2016). Finally, the activity of pathogenic bacteria such as *Salmonella* Thompson might lead to food poisoning (Friesema et al., 2014).

Correct handling and storage of the fish are essential to reduce microbial spoilage. Sterile muscle should be separated from non-sterile parts of the fish at slaughter to limit contamination. Furthermore, contamination from humans and equipment handling the raw material must be limited by hygienic procedures. Low temperatures and lowered water activity by freezing or salting remain some of the most effective methods to decrease the rate of microbial proliferation (Falch et al., 2006).

2.4.4. Lipid Oxidation

Lipid oxidation is the process where unsaturated lipids break down into a diverse mixture of lipid hydroperoxides and small organic compounds in the presence of oxygen. The end products of lipid oxidation cause the off-flavours associated with rancidity. Fatty fishes like salmon are especially susceptible to lipid oxidation since they are rich in polyunsaturated fatty acids (PUFAs) (Sohn & Ohshima, 2010). However, lipid oxidation in salmon is slowed by the antioxidant properties of astaxanthin (J. Lerfall, 2015). Furthermore, lipid oxidation is usually not a problem during the storage of salmon at temperatures above the freezing point because autolysis and microbial activity deteriorate the salmon long before autooxidation. However, lipid oxidation becomes a problem during the long-term storage of frozen fish since the process continues as temperatures below the freezing point (Aydin & Gokoglu, 2014).

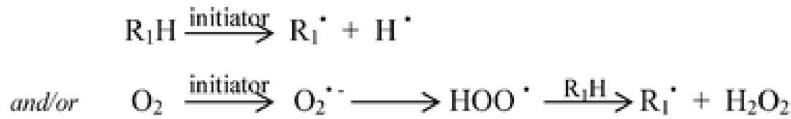
Since lipid oxidation is central when discussing the deterioration of salmon during frozen storage, the main mechanisms of autooxidation will be briefly discussed. Figure 2.7 shows a simplified overview of the three main processes of autooxidation, namely initiation, propagation, and termination.

The initiation step starts when a hydrogen atom is removed from the lipid to form a lipid radical (R·). PUFAs with two methylene interrupted double bonds form a resonance stabilized lipid radical, which lowers its formation energy. Furthermore, the hydrogen atom of the methylene group between two double bonds has a lower C-H bond strength, making it easily removed. Therefore, PUFAs are extra susceptible to lipid oxidation (Shahidi & Zhong, 2009).

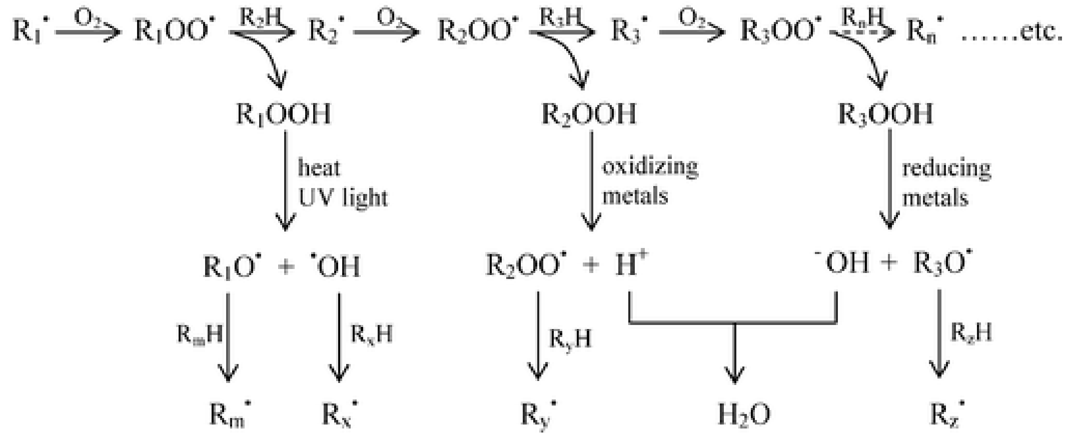
In the first step of propagation, a lipid radical (R·) reacts with oxygen (O_2) to form a lipid peroxyl radical (ROO·). The peroxyl radical then starts a chain reaction, where various compounds are formed. The process is terminated when two lipid radicals react together to form a non-radical product (Shahidi & Zhong, 2009).

Primary oxidation products refer to the lipid hydroperoxides formed in the propagation phase of autooxidation. The formation of these compounds is the rate-limiting step of autooxidation. The main methods to inhibit the formation of primary oxidation products are to isolate the lipids from oxygen, adding antioxidants, or storing the lipids in darkness and at low temperatures (Shahidi & Zhong, 2009). Secondary oxidation products are the various organic compounds produced after the termination step. Among these are alcohols, aldehydes, and ketones. The secondary oxidation products produce the characteristic off-flavour associated with rancidity (Sohn & Ohshima, 2010).

Initiation:



Propagation:



Termination:

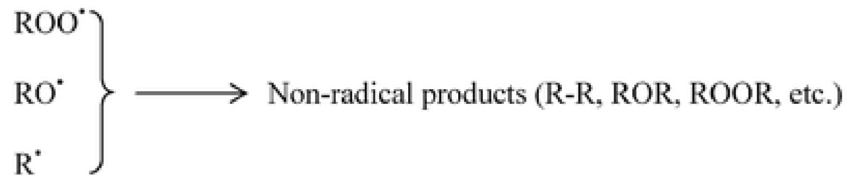


Figure 2.7. Pathway for the initiation, propagation, and termination of lipid autooxidation. Adapted from "Lipid oxidation and improving the oxidative stability" by Shahidi & Zhong (Shahidi & Zhong, 2009).

Methods used for measuring lipid oxidation can be divided into methods that measure primary or secondary oxidation products (Shahidi & Zhong, 2005). One method used to measure primary oxidation products is the peroxide value (PV) (AOCS Official Method Cd 8-53). The PV measures the amount of hydroperoxides in oil found by iodometric titration. Secondary oxidation products can be measured by the thiobarbituric acid (TBA) reactive substances (TBAR) method (Dasgupta & Klein, 2014). TBAR measures the amount of the secondary oxidation product malondialdehyde (MDA) that reacts with TBA to form TBAR. The concentration of TBAR can be measured by spectrometry at 532-535 nm or by chromatography. A combination of methods that measure primary and secondary oxidation products should be used to measure lipid oxidation.

2.5. Methodological Theory

The following section will introduce the most important methods used in this thesis, as well as the theory behind them. It will also serve to clarify why certain methods was chosen over others, and choices made during optimization of laboratory protocols.

2.5.1. Conversion Factors for Protein Determination by the Kjeldahl Method

The Kjeldahl method is one of the most common methods used to determine the protein content in a biological sample, along with the Lowry method, Bradford protein assay, and amino acid determination. This study will use the Kjeldahl method to determine the protein content of salmon co-products, filets, and protein extracts. The Kjeldahl method was chosen instead of the Lowry method or Bradford protein assay since it is the established method in the laboratories of Akrinn, Kalvskinnet, and is known to yield reliable results.

The Kjeldahl method is an indirect protein determination method that determines total nitrogen content through titration. The nitrogen content is converted to protein content through a conversion factor, where 6.25 is the most common. The conversion factor is based on two assumptions; that the average nitrogen content in protein is 16%, and that all nitrogen in food is protein-bound. However, food that contains nitrogen compounds such as nitrate, ammonia, urea, nucleic acids, free amino acids, and alkaloids has also non-protein bound nitrogen (Mæhre et al., 2018). The presence of non-protein bound nitrogen in foods makes a general conversion factor for all foods unspecific.

Mære et al. (2018) found that the protein content in fish is more accurately determined by using a specific conversion factor of 5.6 instead of the general conversion factor of 6.25. They found that the specific conversion factor still overestimated the amount of protein in fish when compared to the protein content determined by amino acid analysis. However, it is a chance that amino acid analysis underestimates the amount of protein in foods due to the complete denaturation of amino acids leading them to conclude that 5.6 is suited as a specific conversion factor for fish.

A disadvantage of using the specific conversion factor of 5.6 is that most studies on salmon use the general conversion factor of 6.25 (Aas et al., 2019; Atanasoff et al., 2013; Mæhre et al., 2018). Using the specific conversion factor for fish would make it challenging to compare results from this study with other studies. Thus, the general conversion factor of 6.25 will be used in this study even though it is likely to overestimate the protein content.

2.5.2. Lipid Extraction by Bligh and Dyer

The extraction method of lipids developed by Bligh & Dyer (1959) utilizes the monophasic and biphasic attributes of a mixture of chloroform, methanol, and water at different ratios. The sample is homogenized in a chloroform, methanol and water solution in the ratio of 1:2:0.8, respectively. The system is monophasic in this ratio, allow the extraction of the lipids into the solution. The system is then made biphasic in a chloroform, methanol, and water ratio of 2:2:1.8, with a chloroform phase and a water-methanol phase. The extracted lipids will be solved in the chloroform phase, while hydrophilic compounds are solved in the water-methanol phase. The lipids can then be isolated by evaporating the chloroform phase. In addition to extracting lipids for further analyses, the method is used to determine the lipid content in samples by weighting the isolated lipids.

2.5.3. Fatty Acid Profile by Gas Chromatography

Gas chromatography (GC) is one of the most common methods used to determine the fatty acid (FA) profile of an oil (Dorman et al., 2010). Longer lipids are more retained than shorter lipids in the chromatography column which split the lipids according to size. Each lipid is measured with an FID-detector, resulting in a chromatogram. The concentration of a FA corresponds to the area under the chromatogram peak. The FA that produces each top of the gas chromatogram is identified by comparing them to the chromatogram of the known standard. The FA profile yields information about the nutritional value of salmon co-products, including omega-3 FAs like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

2.5.4. Distribution and Solubility of Fish Proteins

The water-soluble protein (WSP) of fish muscle is sarcoplasmic proteins dissolved in the cytosol (Mackie, 1993). They constitute 20-35% of the proteins in salmon muscle and are mostly enzymes that do not contribute to the textural characteristics of fish. The salt soluble protein (SSP) is myofibrillar protein such as myosin and actin that make up the muscle filaments. 65-75% of the total protein in muscle are myofibrillar proteins that produce the textural properties of fish. Myosin has excellent gel formation properties and is therefore useful in the production of processed mince products. The last protein group is the insoluble connective tissue that makes up 3-10% of the protein in fish muscle.

2.5.5. Determination of General Proteolytic Activity

General Proteolytic Activity (GPA) is a measure on the activity of all proteases in a sample, in contrast with methods that measure the activity of specific proteases such as cathepsins, trypsin or collagenases. The determination of GPA has three main steps, and will be done according to Barrett (1972) as described by Stoknes et al. (1993). First, endogenous protases are extracted from Salmon. Secondly, the proteases are incubated in suitable conditions,

together with a substrate that the proteases break down. Finally, the amount peptides soluble in Trichloroacetic acid (TCA) in the incubated sample are measured by the Lowry method.

Suitable pH, Temperature and Buffer for Protease Incubation

Incubation time, temperature, and pH are variables with a substantial effect on enzyme activity. The goal of this experiment is to obtain a comparable measure of protease activity between the salmon fractions. Therefore, conditions that produce a high protease activity are desirable. A previous study on proteases in cod shows that proteases from cod Bits & Pieces and filet are highly active at 35 °C, pH 3 (Sovik & Rustad, 2005). Other studies on salmon filets show that alkaline proteases are most active at 60-65 °C, pH 6-9, while acid proteases are most active at 50 °C, pH 3.5-5 (Stoknes & Rustad, 1995).

In this thesis, different incubation conditions were tried out before incubation at 60 °C, pH 6 for 60 min was found to yield acceptable results. pH 6 was chosen to mimic the conditions of the enzymes during the storage of minced salmon (Mørkøre et al., 2010b). Finally, a citric acid-phosphate buffer (pH 6) (*Mc Ilvaine, 1921*) was chosen for the incubation, as it is readily made with a desired pH and salt concentration. It is essential that the buffer does not contain thiol groups that would produce an interfering blue colour during the Lowry method.

General Proteolytic Activity Increase the Amount of Peptides Soluble in TCA

TCA increases the hydrophobicity of proteins, leading to aggregation through hydrophobic interactions. Aggregation decreases protein solubility, causing proteins to precipitate. Long peptide chains are less soluble in TCA than short peptide chains. Yvon et al. (1989) found that peptides soluble in 10% TCA had an average size of 330-380 Da (3-4 residues). However, peptides and proteins solubility in TCA is also affected by the amino acid composition. During incubation of proteases in the GPA analysis, proteases cleave haemoglobin to produce short-chained peptides. Thus, GPA increases the amount of TCA soluble peptides in the solution. To assess the GPA, a blank sample is prepared by adding TCA before incubation. The difference in TCA soluble peptides between the incubated sample and the blank sample becomes a measure of protease activity. The GPA are then described as the amount of TCA soluble peptides liberated during incubation per gram of salmon in the protease extract per hour of incubation.

The Amount of TCA Soluble Peptides Will be Measured by the Lowry Method

Along with Kjeldahl and Bradford, the Lowry method is one of the most common ways to determine protein content in a sample (Mæhre, 2018). The method is based on the ability of the Folin-Ciocalteu reagent to produce a blue-coloured complex with peptides in an alkaline cobber solution. The resulting solution will absorb light at 750 nm. The measured absorbance at 750 nm is converted to peptide concentration by a standard curve produced with Bovine Albumin Serum (BSA) solutions of known concentrations.

2.5.6. Acid Value

The acid value, or acid number, is defined as the amount of KOH in milligrams required to neutralize 1 gram of an oil (ISO.660, 2020). The acid value describes the amount of FFAs in an oil. FFAs are produced by the breakdown of triglycerides which is catalysed by lipases, both endogenous from the fish and by microbial lipases (Koczoñ et al., 2008). This process facilitates lipid oxidation, as FFAs are more readily oxidized than triglycerides (Shahidi & Zhong, 2009). The acid value increase early in the oxidation process as FFAs are produced from triglycerides. The acid value is used as an indication of oil quality (FAO, 2017) and as an indirect measure of the lipase activity in the oil (Cong et al., 2020).

2.5.7. ATP degradation by High Performance Liquid Chromatography

The concentrations of Hx, IMP, and Ino can be determined by High-performance liquid chromatography (HPLC). The concentrations of ATP, ADP and AMP will not be analysed in this study as they quickly deteriorate *post-mortem* (Wang et al., 1998). A standard of known concentrations of Hx, IMP and Ino are used to produce a standard curve. The standard curve identifies the relationship between the area under each peak in the chromatogram and the concentrations of each substance. The concentrations of Hx, Imp, and Ino are used to calculate the Ki-value, which yields information about the sensory properties of the samples (Saito et al., 1959).

2.6. Prototype Experiments

In the early work of this thesis, considerable time was invested in learning and optimizing the laboratory methods. All analysis used in the thesis has first been performed with a small sample size. Some of the experiments yielded poor or no results and had to be repeated with modifications until satisfactory continuity in the results was obtained. Furthermore, the work of this thesis has been delayed due to the COVID-19 pandemic. Access to the lab has been restricted in some periods, and access to supervisors and laboratory engineers has been reduced.

The measure of GPA in particular was a time-consuming analysis. No personnel on the lab were familiar with the analysis, which meant the methodology had to be developed from scratch. The main procedure was found through a literature review and with the kind help of professor Turid Rustad. The optimization of the procedure mainly concerned buffers, ratios, and filtration for the protease extraction, pH, and temperature for the incubation and dilutions for the Lowry analysis. The experiment was modified and repeated by trial and error a total of eight times, which resulted in a 10-100-fold increase in GPA and more consistent results between experimental parallels (Appendix 1, Table A-1) (Appendix 1, Table A-2).

The Bligh & Dyer lipid extraction method used to find the lipid content was another time-consuming method to develop. In contrast with the GPA analysis, laboratory protocols were available. The main challenge with the method was to achieve similar results in experimental parallels. The experiment had to be modified and repeated four times before satisfactory continuity between parallels was achieved. Furthermore, it was challenging to extract enough lipids for the acid number and FA profile analysis. This problem was solved by increasing the scale of the extraction.

In the first experiments, pieces of salmon filets and co-products were used for analyses instead of mince. The use of pieces made it challenging to differentiate what observed variations were caused by actual differences between Sup and Prod quality salmon or co-product fractions, and what was caused by individual variety between salmons. Furthermore, differences within the same salmon fraction, such as the varying lipid content of the filet fraction, caused further interference (Katikou et al., 2001). The uncertainties caused by varying composition within each fraction and variation between individual salmons was reduced by using pooled samples, where muscle from 5 individuals was minced together in one batch. Another advantage of using mince is that it replicates the conditions in the industry, where salmon co-products are used to produce frozen mince blocks that can be further processed (Olsen & Johnsen, 2003).

3. Materials and Methods

3.1. Experimental Design and Sample Preparation

A flowchart of the experimental design is presented in Figure 3.1.

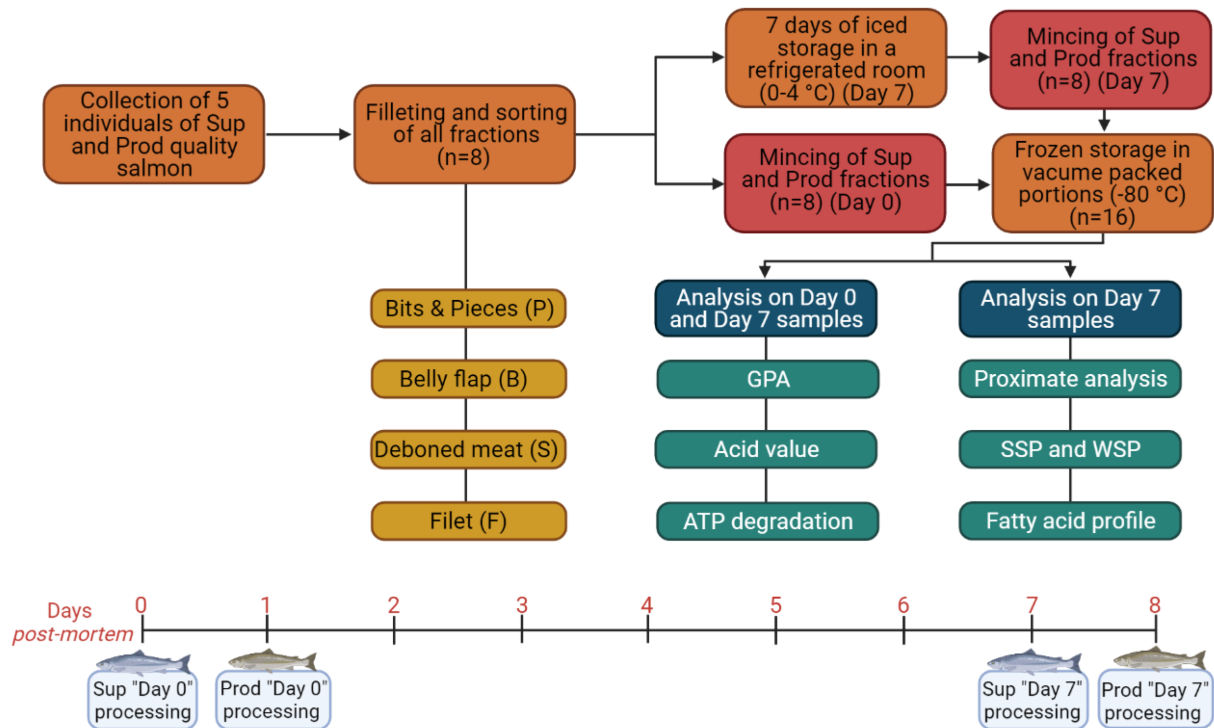


Figure 3.1. Flowchart of the experimental design and timeline for the processing of Sup and Prod in *days post-mortem*. The figure includes denotations used in the thesis when referring to different salmon fractions (P, B, S, and F), storage groups (Day 0 and Day 7), and qualities (Sup and Prod). The figure was made by using the online software available at BioRender.com. Sup= Superior; Prod = Production; GPA= General proteolytic activity; SSP= Salt-soluble protein; WSP = Water-soluble protein. T

Ten salmon were collected, five of Sup quality and five of Prod quality. The fish were filleted and sorted into the fractions used in the experiment. The co-product fractions are the Bits & Pieces (P), belly flaps (B), and deboned meat (S) from the backbone (Figure 3.2) (Figure 3.3). In addition, we chose to use a slice of the front, middle, and back of the top and middle loin for analysis of the fillet (F).

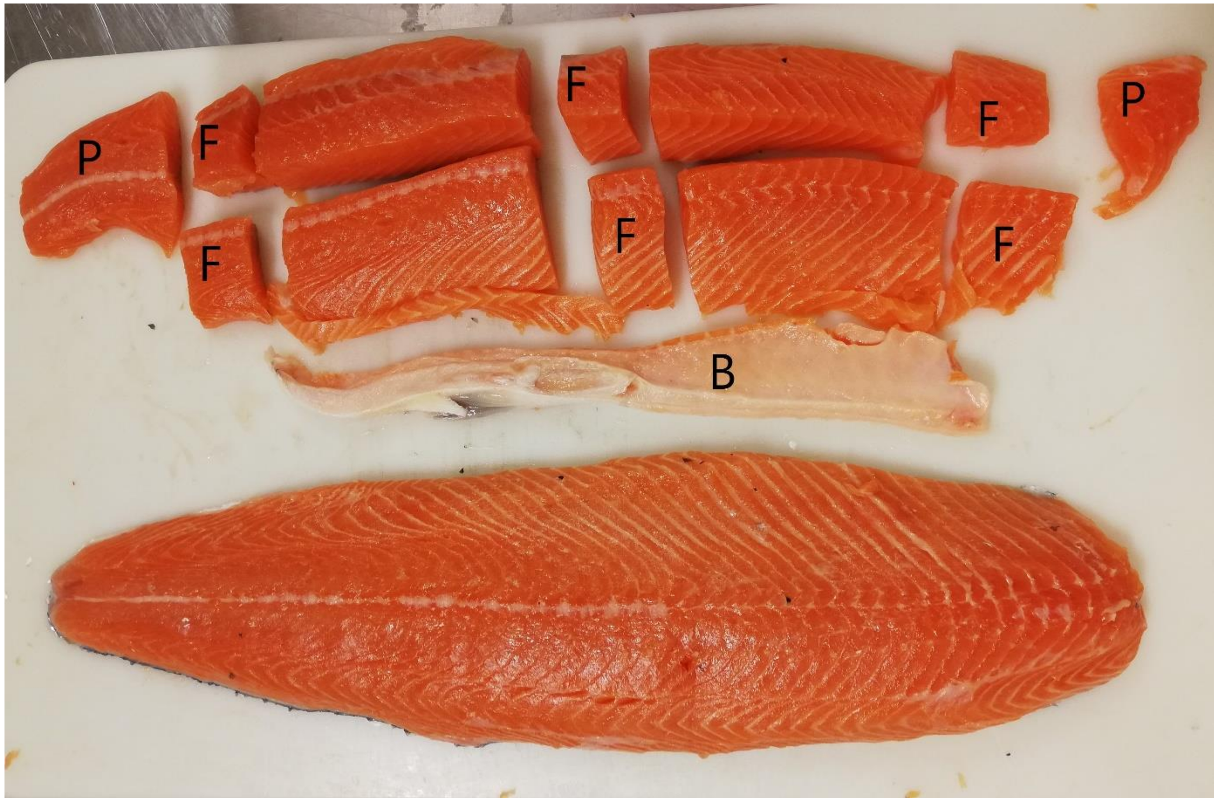


Figure 3.3. Illustration of the fractions used in the study. The bottom fillet is uncut, while the upper are cut in the way the fractions are used in this study. The belly flap in the picture originates from the bottom filet. The letters illustrate which fractions the different pieces are defined as, where F= fillet, P= Bits & Pieces, B= belly flap.

Figure 3.2. Production of deboned meat used in the study. Industrially, the meat is deboned using a machine. In this study, it was deboned manually using a spoon, as shown. Only red meat was removed, while the soft, fatty tissue visible in the upper part of the backbone is left on the frames.

With four fractions (P, B, S, and F) and two qualities (Sup and Prod) the process resulted in eight sample batches, where each batch contained meat from five individual salmons. Each of the batches was divided into two parts. One part was minced and frozen (-80 °C) in vacuum-packed portions immediately, hereby referred to as the Day 0 samples. The other part was stored on ice in a refrigerated room (0-4 °C) in open plastic bags for 7 days, hereby referred to as Day 7 samples. After 7 days, the samples were minced and frozen (-80 °C) in vacuum-packed portions. All fractions were minced using a Blixer 6 mincer (Robot Coupe, France), and vacuum packed using a SuperMax-C vacuum packaging machine (Webomatic, Germany).

All analysis was performed on both Sup and Prod quality Atlantic Salmon. Day 7 samples were used for the proximate analysis, determination of salt- and water-soluble protein, and determination of the FA profile. Meanwhile, both Day 0 and Day 7 samples were used to determine general proteolytic activity, acid value, and ATP degradation.

The Sup quality salmon was collected freshly slaughtered directly from the slaughtering facility. Meanwhile, the Prod quality salmon were first slaughtered at one location before it was collected from a processing facility the next day. It must be stressed that the Prod quality salmon was slaughtered one day prior to collection. The time delay before collection of the Prod quality salmon means that that the "Prod Day 0" samples actually are one day *post-mortem*, while the "Prod Day 7" samples actually are eight days *post-mortem*. A timeline showing the *post-mortem* processing times for the salmon samples is included in Figure 3.1.

3.2. Origin of Raw Material

Five whole, gutted, and newly slaughtered Atlantic salmon (*Salmo salar* L.) of Sup quality was collected at Lerøy AS, Jøsnøya 26.11.2020. The salmon was transported in polystyrene boxes with ice to the food processing lab at NTNU, Akrinn, Trondheim. Arriving at the Lab, each fish was immediately treated as previously described in the chapter above (Figure 3.1).

The procedure was repeated with Prod quality salmon collected at Isfjord AS, Orkanger 4.12.2020. The Prod fish was slaughtered at Sinkaberg Hansen AS, Rørvik, 3.12.2020.

3.3. Proximate Composition

A complete proximate analysis was performed on Day 7 samples of all four fractions (P, B, S, and F) of both Sup and Prod quality salmon. The proximate composition includes the determination of protein, lipid, water, and ash content in the salmon.

3.3.1. Protein Determination by the Kjeldahl Method

The protein content in the salmon mince was determined according to method 981.10 of the AOAC International (Latimer, 2016).

1-2 g of salmon mince was accurately weighted out on Kjeldahl papers and placed in 750 mL sample tubes together with 2 Kjeldahl catalyst tablets and 15 mL 97% sulfuric acid (H_2SO_4). A blank sample and a control sample with 0.1-0.2 g of 99.7% glycine were included in each run. Before digestion, the heating block was preheated to 280 °C. The samples were heated at 320 °C for 20 min and 420 °C for 90 min. Thereafter, the fully digested samples were titrated with a KjelMaster K-375 (Büchi, Switzerland) to determine total nitrogen in the samples. The percentage protein in each sample was calculated with Equation IV.

$$\%Protein = \frac{N(g) * 6.25}{sw\ (g)} * 100\% \qquad \text{Equation IV}$$

Where *N* is the weight of nitrogen in the sample, 6.25 is the Kjeldahl conversion factor, and *sw* is the wet weight of salmon in the sample. All samples were analysed in triplicates.

3.3.2. Water and Ash Determination

The water and ash content in the samples were analysed according to the ISO standard ISO.6496, (1999).

3-4 g salmon mince was weighted into crucibles of known weight. The crucibles were heated in an oven at 105 °C for 22-24 hours, allowing all the water to evaporate. After heating, the samples were cooled down in a desiccator before they were weighted again. The water content in each sample was calculated using Equation V.

$$\% \, Water = 1 - \frac{w2(g)}{w1(g)} * 100\% \qquad \text{Equation V}$$

Where *w1* is the weight of the crucible and sample before drying, and *w2* is the weight of the crucible and sample after drying.

To determine the ash content, the dried samples were heated in a muffle furnace for 22-24 hours at 550 °C. The samples were then cooled down in a desiccator and weighted again. The ash content in each sample was calculated using Equation VI.

$$\% \, Ash = 1 - \frac{w3(g)}{w1(g)} * 100\% \qquad \text{Equation VI}$$

Where *w1* are the weight of the crucible and sample before drying and *w3* are the weight of the crucible and sample after heating in the muffle furnace. All samples were run in triplicates, and the analysis was preformed two times.

3.3.3. Lipid Determination by Bligh & Dyer

The lipid content in the salmon mince was determined using a modification of the method described by Bligh & Dyer (1959).

5-10 g of minced salmon, 16 mL distilled water (diH_2O), 40 mL methanol (CH_3OH), and 16 mL chloroform ($CHCL_3$) were added to 200 mL centrifugation tubes. The resulting samples were homogenized with an Ultra Turrax T-25 for 2 min before 20 mL $CHCL_3$ was added. Moreover, they were homogenized for 20 s before 20 mL diH_2O was added, and the samples were finally

homogenized for another 20 s and centrifuged (3000 g, 15 min, 4 °C). The chloroform phase containing the lipids was then pipetted into a separate container.

2 mL of the isolated chloroform phase was pipetted into weighted reagent glasses, with two parallels for each sample. The chloroform was allowed to evaporate under nitrogen gas for 15 min, and the samples were cooled in a crucible before weighting the lipids. The weighting was done in triplicates for each extract, with between 3 and 5 extracts for each sample. The content of lipids in the sample is determined by weight after Equation VII.

$$\%Lipid = \frac{Tube\ with\ lipid\ (g) - tube\ without\ lipid\ (g) * V_{CHCl l_3}(total)(mL)}{sw\ (g) * V_{CHCl_3}(isolated)(mL)} * 100\% \qquad \text{Equation VII}$$

Where $V_{CHCl l_3}(total)$ is the total volume of chloroform added, $V_{CHCl_3}(isolated)$ is the volume of chloroform that was isolated and evaporated in a weighted tube, and *sw* is the wet weight of the salmon in the sample.

The remaining lipid phase was frozen down at -80 °C in a nitrogen gas atmosphere and subsequently used in the acid value and FA profile analyses.

3.4. Water- and Salt-soluble Protein

The water-soluble protein (WSP) and salt-soluble protein (SSP) were extracted using a modification of the method described by Anderson & Ravesi, (1986). The WSP was extracted with Buffer 1 (0.05 M Monopotassium phosphate (KH_2PO_4), pH 7). The SSP was extracted with Buffer 2 (0.05 M KH_2PO_4, 0.6 M Potassium chloride (KCl), pH 7).

4 g accurately weighted salmon mince and 50 mL Buffer 1 was homogenized using an Ultra-Turrax T25 for 10 s. The samples were centrifuged (20min, 3000g, 4 °C) thereafter, and the supernatant was decanted into a separate tube through filter paper. The resulting filtrate is the WSP fraction. The precipitate was then resuspended in 50 mL Buffer 2, and the process was repeated. The resulting filtrate is the SSP fraction.

This process was repeated with mince from all four fractions of Sup and Prod quality salmon. The experiment was done with triplicates of all extractions, and the extract was frozen at -80 °C until protein determination.

The amount of nitrogen in the WSP and SSP extracts was determined with the Kjeldahl method, with two modifications to the method previously described. Firstly, 16-20 mL of the WSP and SSP extracts was accurately pipetted into Kjeldahl tubes instead of 1-2 g mince. Secondly, the digestion time and temperature were altered to allow the liquid in the extracts to evaporate. Before digestion, the heating block was preheated to 150 °C. The samples were then heated at 170 °C for 15 min, 220 °C for 30 min, and finally 420 °C for 100 min. The amount of WSP and SSP protein in each sample was calculated with Equation VIII.

$$\%Soluble\ protein = \frac{N(g/L) * 6.25}{B(L) * sw\ (g)} * 100\% \qquad \text{Equation VIII}$$

Where *N* is the concentration of nitrogen in the extract, 6.25 is the Kjeldahl conversion factor, *B* is the volume of buffer in the extract, and *sw* is the wet weight of salmon in the extract. All extracts were analysed in duplicates.

3.5. Fatty Acid Profile by Gas Chromatography

The lipids extracted by the Bligh & Dyer method were analysed by Gas Chromatography (GC) as fatty acids (FA) methyl– esters. The FA methyl-esters were prepared as described by Metcalfe et al. (1966).

0.1-0.6 g of lipid and 3 mL potassium hydroxide (KOH) (0.5 M) in methanol was mixed with a vortex mixer before heating in a water bath at 70 °C for 20 min. The solution was stirred with a vortex mixer several times during the heating. It was then cooled on ice before the addition of 5 mL Bromine trifluoride (BF_3) (13% v/w) in methanol and heated at 70 °C for another 5 min. After heating, the lipids were cooled on ice before the addition of 2 mL n-butyl acetate ($C_6H_{12}O_2$). The sample was stirred, and saturated sodium chloride (NaCl) solution was added until the liquid level was 1 cm from the top of the sample glass. Anhydrous sodium sulphate (Na_2SO_4) was gently sprinkled through the sample before the lipid phase was pipetted into a separate tube. Finally, all samples were filtered once through a 0.2 µm nylon syringe filter.

1 µL of the resulting FA methyl esters was injected into an Agilent 6850 GC-system (Agilent Technologies, USA) equipped with a polyethylene glycol capillary column (HP-INNOWax) 30 m × 250 µm×0.25 µm and a flame ionization detector (FID, 310 °C). Helium was used as the carrier gas, and the oven had an isothermal temperature of 210 °C. The resulting chromatogram was analysed using a lipid standard as a reference. The analysis was performed once for each salmon fraction.

3.6. General Proteolytic Activity

The general proteolytic activity (GPA) of endogenous proteases was analysed by extracting the enzymes, incubating them, and then analysing the content of peptides soluble in 2.5% TCA ($C_2HCl_3O_2$) by the Lowry method. The analysis was performed on both Day 0 and Day 7 samples to measure the change in protease activity during storage.

3.6.1. Protease Extraction

The proteases were extracted using a modification of the method described by Stoknes & Rustad (1995).

10 g of salmon mince and 20 mL diH_2O was homogenized with an Ultra Turrax T-25 for 2 min, and then shook with an orbital laboratory shaker (MRC laboratory instruments, England) for 30 min. The samples were centrifuged (20 min, 3000g, 4 °C) before the supernatant was filtered through a filter paper. The protease extracts were made in triplicates for all samples and frozen down at -80 °C until use.

3.6.2. Protease Incubation

The protease extracts were incubated according to Barrett (1972) as described by Stoknes & Rustad (1995). A schematic overview of the described methodology is presented in Figure 3.4.

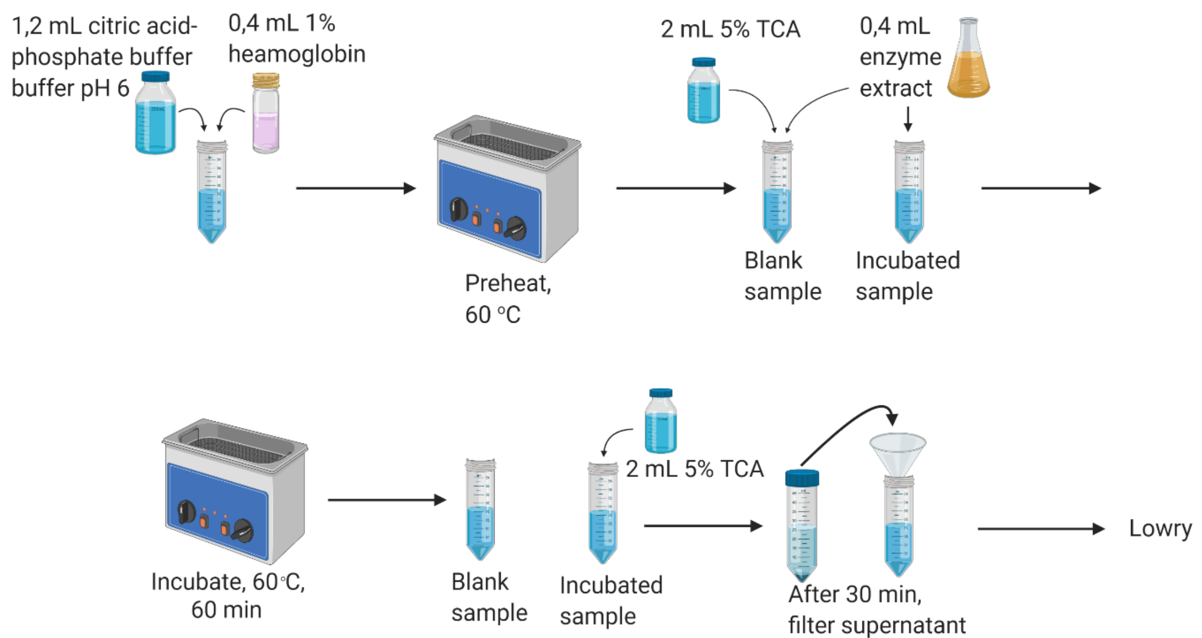


Figure 3.4. Schematic overview of the methodology used in protease incubation. TCA = Trichloroacetic acid. The figure was made by using the online software available at BioRender.com.

1.2 mL citric acid ($C_6H_8O_7$)-phosphate (PO_4^{3-}) buffer (pH 6) and 0.4 mL of haemoglobin substrate (1% w/v) was preheated in a water bath of 60 °C. Additionally, 2 mL TCA (5% v/w) was added to the blank samples prior to incubation. 0.4 mL of the protease extract was then added to all samples, and the samples were left to incubate in a water bath for 60 min at 60 °C after mixing with a vortex mixer. 2 mL TCA (5% v/w) was added to the incubation samples after precisely 60 min and the samples were mixed. The incubation mixtures were cooled down for 30 min and subsequently filtered through filter paper.

The samples were left in a refrigerator overnight before the amount of TCA soluble peptides was analysed by the Lowry method the next day. All analysis was done in triplicates for the blank samples and the incubation samples, resulting in a total of 6 samples for each protease extract.

3.6.3. Peptide determination by the Lowry Method

The concentration of peptides soluble in TCA (2.5% v/w) was determined with a modification of the method described by Lowry et al. (1951). Prior to the experiment, a series of solutions were prepared (Table 3-1). Additionally, a dilution series of bovine serum albumin (BSA) was used to produce a standard curve of know protein content. A new reagent D, diluted Folin reagent, and BSA standard curve was made for each new day the protein content of the incubated protease extracts was measured. A schematic overview of the described methodology is presented in Figure 3.5.

Table 3-1. Ingredient list of the solutions prepared for the Lowry protein determination.

Solution	Ingredients
A	Na_2CO_3 (2% v/w) in NaOH (0.1 M)
B	$CuSO_4$ (1% v/w) in distilled H_2O
C	$KNaC_4H_4O_6$ (2% v/w) in distilled H_2O
D	150 mL Solution A, 1.5 mL solution B and 1.5 mL solution C
Diluted Folin reagent	4 mL Folin-Ciocalteu reagent, 8 mL distilled H_2O

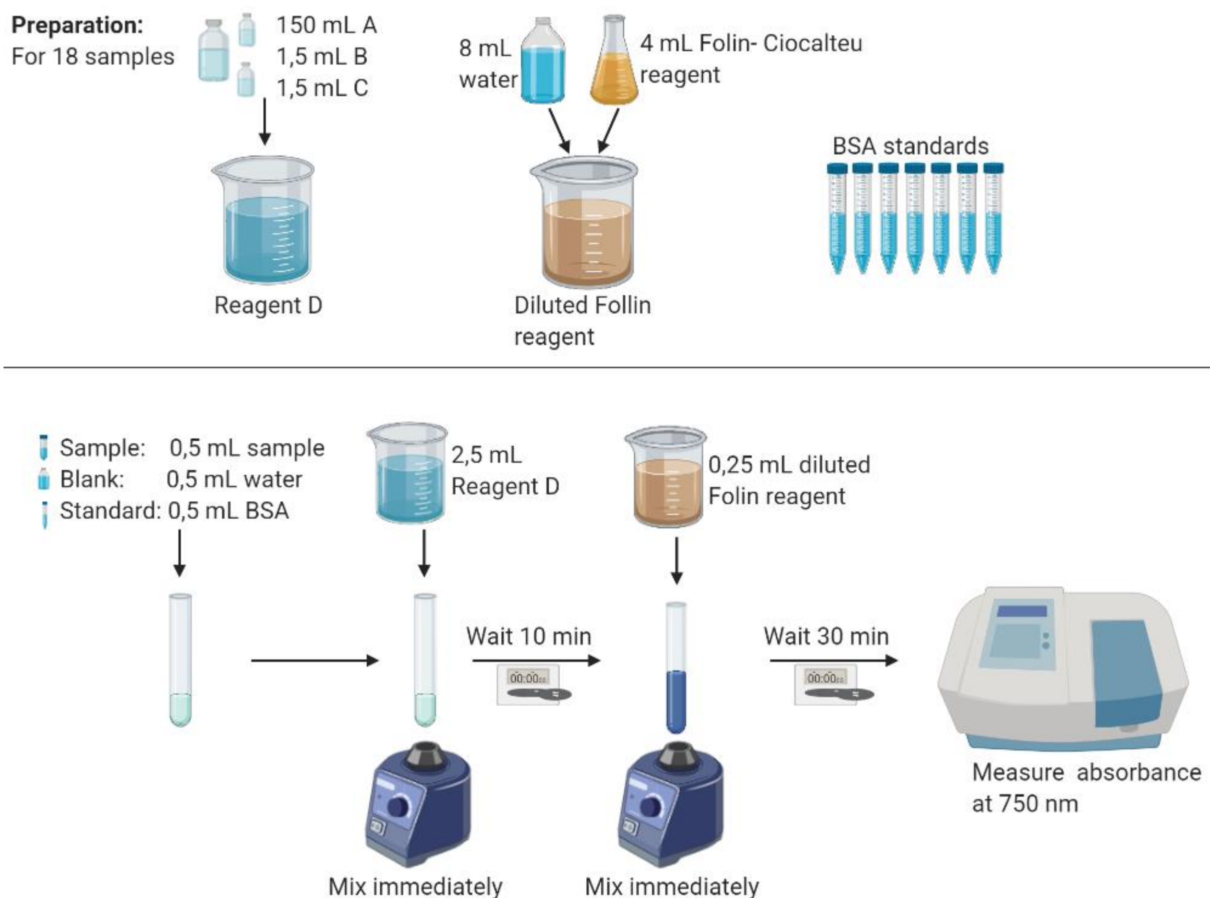


Figure 3.5. Schematic overview of the methodology used analyze the protein content of the samples using the Lowry method. BSA = Bovine Serum Albumin. The figure was made by using the online software available at BioRender.com.

0.5 mL of incubated protease sample, blank (distilled water) or BSA standard was mixed with 2.5 mL of reagent D and left for exactly 10 min. 0.25 mL of diluted Folin reagent was added to the sample, and the sample was mixed. After exactly 30 min, the absorbance was measured at 750 nm. The concentration of peptides in the incubated samples was determined based on the measured absorbance using a standard curve produced with the BSA standards. The amount of TCA soluble peptides in the enzyme extracts per wet weight salmon was then calculated using Equation IX.

$$TCA_e\ (mg)/\ sw\ (g) = \frac{TCA_i(mg/mL) * V_i(mL) * V_e\ (mL)}{V_{ie}(mL) * sw\ (g)} \qquad \text{Equation IX}$$

Where TCA_e is the weight of TCA soluble peptides in the enzyme extract, *sw* is the wet weight of salmon in the enzyme extracts, TCA_i is the concentration of TCA soluble peptides in the incubated sample, V_i is the volume of the incubated sample, V_e is the volume of the solvent in the enzyme extract, and V_{ie} is the volume of the enzyme extract added to the incubation mix. Each sample was analysed one time, as they are already in triplicates from the incubation step.

3.7. Acid Value by Titration

The acid value of the salmon oil extracted with the Bligh and Dyer method was determined according to ISO.660 (2020). The analysis was performed on lipids from both Day 0 and Day 7 salmon.

The samples were prepared by making a monophasic solution of 8 mL $CHCL_3$, 4 mL CH_3OH, and 8 mL isopropanol (Iso-C_3H_8O), containing 0.1-1 g lipids. The acid value was found by acid-base titration, using 0.05 M NaOH as a base and 0.5% Meta Cresol purple as an indicator. A blank sample was prepared by excluding oil from the sample. The acid value was calculated with Equation X.

$$Acid\ value = \frac{(T(mL) - B(mL)) * 1.41}{S(g)} \qquad \text{Equation X}$$

Where *T* is the titrated volume of 0.05 M NaOH in each sample, *B* is the titrated volume of 0.05 M NaOH in the blank sample, 1.41 is a conversion factor from NaOH to KOH and *S* are the weight of oil in each sample. The samples were analysed in duplicates.

3.8. ATP degradation by High Performance Liquid Chromatography

The concentrations of IMP, Ino, and Hx in Day 0 and Day 7 samples were determined by high-performance liquid chromatography (HPLC). These concentrations were used to calculate the Ki-value and H-value of the samples using Equations II and III, respectively, presented in section 2.4.2.

ATP degradation compounds were extracted by solving 1.2 g weighted salmon mince in 7.5 mL TCA (7.5% w/v). The samples were homogenized using an Ultra Turrax T-25 for 60 s before centrifugation (20 min, 3000 g, 4 °C). Subsequently, the supernatant was filtered through a filter paper. The extracts were then filtered twice through a 0.2 µm nylon syringe filter before they were frozen at -80 °C until analysis. The extracts were made in triplicates for each sample.

The extracts were analysed using an Agilent 2190 HPLC system (Agilent Technologies, USA) equipped with an infinity diode array detector and a Poroshell 120 column (EC-C18 3,0 x 100mm, pore size 2,7µm). The mobile phase consisted of 0.215 M monopotassium phosphate (KH_2PO_4) and 0.0023 M Tetrabutylammonium hydrogen sulphate ($[CH_3(CH_2)_3]_4N(HSO_4)$) in 3.5% acetonitrile (CH_3CN) adjusted to pH 6.25. The column temperature was 20 °C. The flow rate was set to 0.2 mL/min for minute 0-2, 0.8 mL/min for minute 2-9 and 0.2 mL/min for minute 9-10. The resulting chromatogram was analysed using a standard with known concentrations of Imp, Ino, and Hx.

3.9. Statistics

All data and standard curves were processed in Microsoft excel (2017), while statistical analysis was performed in IBM SPSS Statistics v.27. All results are presented as mean values ± standard error ($\bar{x}$ ± SE). The sample size (n) varies between analyses. Outlying results due to known experimental errors were excluded from the dataset. All statistical tests in this study use a significance level of α= 0.05.

A 2-tailed independent samples t-test with a Levene's Test for Equality of Variance was used for a main effect analysis testing for significant differences between Prod and Sup samples and Day 0 and Day 7 samples. Prior to this main effect analysis, all results in the relevant categories were pooled to generate two means that were compared using the t-test. Significant differences between salmon fractions (P, B, S, and F) were tested with a one-way Analysis of Variance (ANOVA) using Tukey's multiple comparisons test for post-hoc. Correlations between measured parameters were analysed with a Pearson correlation test.

4. Results

4.1. Proximate Composition

The proximate composition of the salmon co-products and filet of Sup and Prod quality are presented in (Figure 4.1).

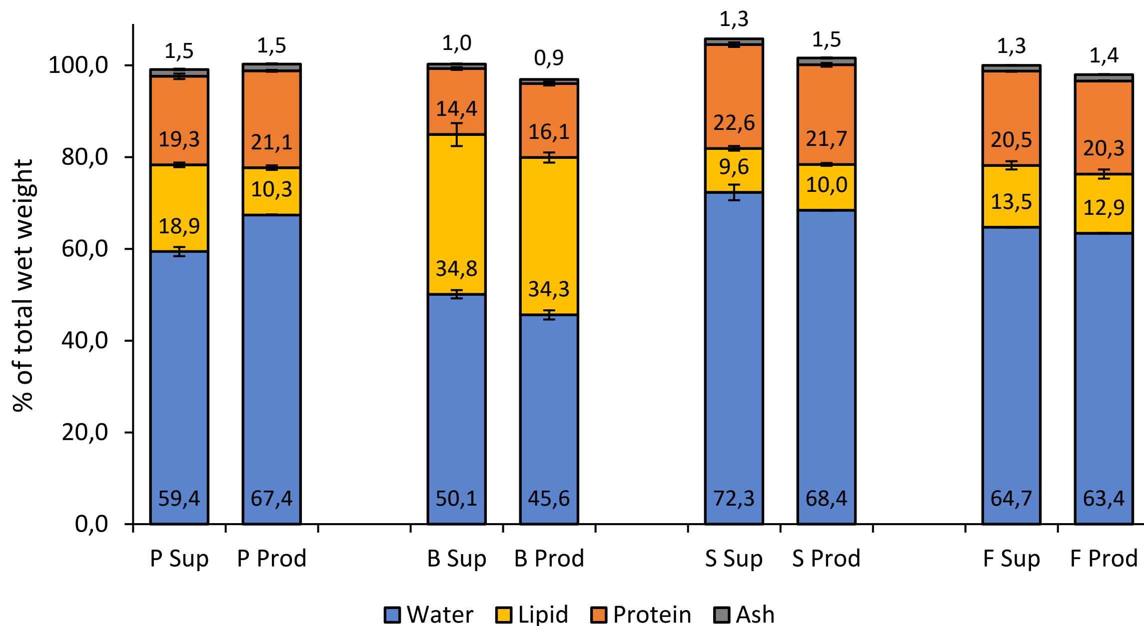


Figure 4.1. Proximate composition of farmed Atlantic salmon. The results are sorted as Superior (Sup) and Production (Prod) quality, and in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S) and Filet (F). All values are presented as the weight fraction of total wet weight, expressed as mean with SE as error bars. The sample sizes of the mean of % water is (n = 5-6), % ash (n = 2-6), % protein (n = 3) and % lipid (n = 3-5).

Differences between Sup and Prod quality salmon was studied with a main effect analysis where data from all the samples in each quality group was pooled. There was found no significant difference in the percentage content of water ($p \geq 0.92$), ash ($p \geq 0.39$), protein ($p \geq 0.60$), or lipid ($p \geq 0.31$) between Sup and Prod quality salmon.

Differences between co-products and the filet were studied by pooling data from Sup and Prod quality for each fraction. The belly flap has significantly higher ($p < 0.001$) lipid content and significantly lower ($p < 0.001$) water and protein content than the other salmon co-products and filet. Meanwhile, the deboned meat is significantly leaner ($p < 0.001$) than the other fractions but is significantly higher ($p < 0.001$) in protein and water. The Bits & Pieces and Filet are between the Belly flap and Deboned meat in protein, lipid, and water content. The ash content is similar in all fractions except for the belly flap that has a significantly lower ($p = 0.004$) ash content than the other co-products.

The differences between the Sup and Prod samples within each fraction illustrate the variations in the raw material. As the values are presented in Figure 4.1, the protein content varies significantly ($p < 0.001$) with approximately ± 2% within each co-product fraction. The lipid content does not vary significantly ($p > 0.05$) within fractions, except for the Bits & Pieces, where the Sup samples have a significantly higher ($p < 0.001$) lipid content than the Prod sample. This difference is interesting, as it illustrates the variation in composition that must be expected in a co-product mince consisting of trimmings from various locations on the fish. Finally, the water content varies significantly ($p < 0.001$) with ± 4-8% within the co-products.

4.2. Water- and Salt-soluble Protein

The amount of WSP and SSP are presented together with the rest protein in Figure 4.2. Rest protein is the non-extractable protein defined as the difference between the total protein found in the proximate analysis and the extracted protein. By pooling data from all fractions in each quality, there was found no significant difference in the content of WSP ($p \geq 0.40$) or SSP ($p \geq 0.30$) between Sup and Prod salmon. The content of WSP was higher than the content of SSP in all fractions. Furthermore, all samples have a high content of non-extractable rest protein.

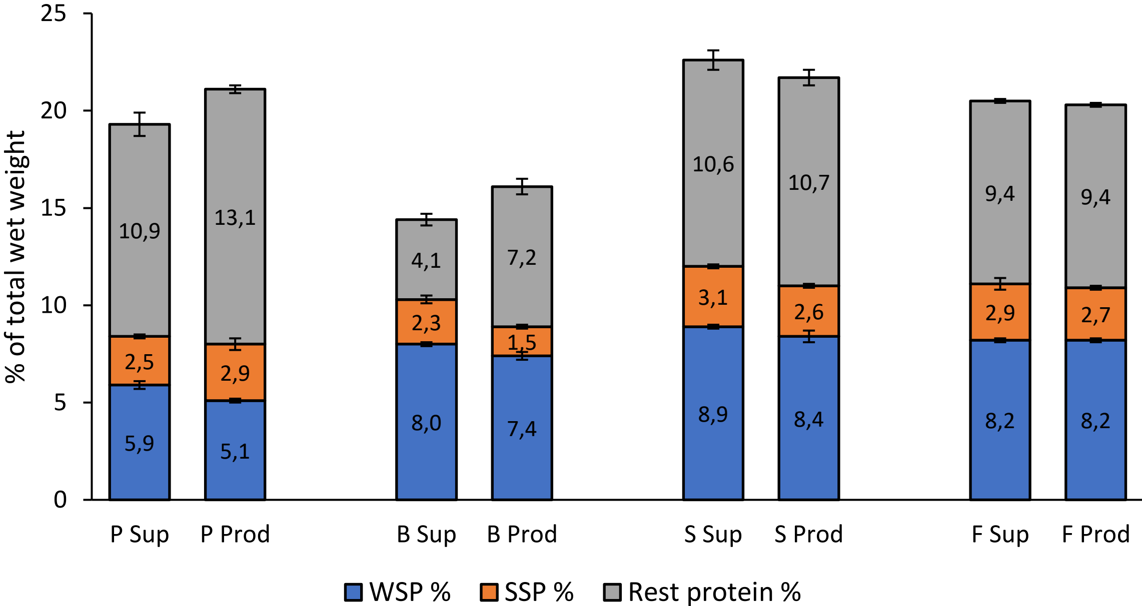


Figure 4.2. Water-soluble protein (WSP), Salt-soluble protein (SSP) and rest protein of farmed Atlantic salmon. The results are sorted as Superior (Sup) and Production (Prod) quality, and in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S) and Filet (F). WSP and SSP are presented as the weight fraction of total wet weight, expressed as mean with SE as error bars (n=3). Rest protein are calculated as *Rest protein % = Total protein % - (WSP% + SSP%).*

The Bits& Pieces fraction has a significantly lower ($p < 0.001$) amount of WSP than the other co-products, while the deboned meat has a significantly higher ($p < 0.001$) amount of WSP

than other fractions. The belly flap is the only fraction that deviates in SSP, with a significantly lower ($p = 0.007$) content.

4.3. Fatty Acid Profile

The FA profile (Table 4-1) was analysed for Sup and Prod samples of all salmon fractions. No statistical analyses are presented since each sample were analysed in only one parallel.

The different FAs are equally distributed in the Prod and Sup quality salmon. Furthermore, the FA profile in the co-products and filet were similar. The Prod quality belly flap and Sup quality deboned meat deviate from other fractions with a slightly higher content of monounsaturated fatty acids (MUFAs) and a slightly lower content of PUFAs. This is mainly caused by stearidonic acid (C18:4n-3) that was not detected in these samples. The co-products and the filet consist of 14-16% saturated FAs, 52-56% MUFAs and 29-31% PUFAs. 12-14% of the lipids are n-3 PUFAs, with an n-3/n-6 ratio of 0.8-1. The most common FA is the MUFA Oleic acid (C18:1n-9), which constitutes of 40-42% of the lipids.

Table 4-1. **Fatty acid profile as proportional content (% of total identified fatty acids) of farmed Atlantic Salmon.** The results are sorted as Superior and Production quality, and in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S), and Filet (F).

	Superior quality				Production quality			
Fatty acid	**P**	**B**	**S**	**F**	**P**	**B**	**S**	**F**
C 14:0	2.1	2.1	1.9	1.9	1.9	2.1	2.0	2.0
C 16:0	9.8	9.8	10.6	10.8	10.8	9.9	10.4	9.9
C 16:1n-7	2.6	2.6	2.4	2.4	2.4	2.7	2.5	2.6
C 18:0	2.9	2.9	3.1	2.9	2.9	2.8	2.8	2.7
C 18:1n-9	41.4	41.6	42.3	40.0	40.0	42.0	40.4	40.8
C 18:1n-7	2.7	2.8	3.1	2.7	2.7	2.9	2.8	2.6
C 18:2n-6	14.5	14.6	14.4	13.6	13.6	14.7	13.7	14.0
C 18:3n-3	7.3	7.4	7.0	5.7	5.7	6.0	5.9	6.1
C 18:4n-3	0.7	0.7	ND	0.8	0.8	ND	0.7	0.7
C 20:1n-9	3.7	3.7	3.8	4.3	4.3	4.4	4.4	4.3
C 20:4n-6	1.0	1.0	1.1	1.1	1.1	1.2	1.1	1.0
C 20:5n-3	2.8	2.6	2.8	2.9	2.9	2.7	3.0	2.8
C 22:1n-9	2.4	2.0	2.7	3.6	3.6	3.6	3.6	3.6
C 22:5n-3	1.2	1.2	1.2	1.2	1.2	1.2	1.3	1.2
C 22:6n-3	3.6	3.6	3.8	4.7	5.0	3.7	4.5	4.3
Σ SAF	14.7	14.7	15.5	14.9	15.6	14.7	15.1	14.6
Σ MUFA	52.8	52.7	54.2	52.8	53.1	56.1	53.7	53.8
Σ PUFA	31.0	31.1	30.2	30.8	30.3	29.1	30.2	30.1
Σ n-3 PUFA	14.3	14.3	13.6	14.1	14.4	12.5	14.2	13.9
Σ n-3 / Σ n-6	0.9	0.9	0.9	0.9	1.0	0.8	1.0	0.9

SAF =saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; ND= Not detected. ΣSAF, (C14:0 + C16:0 + C18:0); ΣMUFA, (C16:1n-7 + C18:1n-9 + C18:1n-7 + C20:1n-9 + C22:1n-9); ΣPUFA, (C18:2n-6 + C18:3n-3 + C18:4n-3 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-6), Σn-3 PUFA, (C18:3n-3 + C18:4n-3 + C20:5n-3 + 22:6n-3); Σn-6 PUFA, (C18:2n-6 + C20:4n-6).

4.4. General Proteolytic Activity

The measured GPA in the co-products and filet of Day 0 and Day 7 Sup and Prod salmon are presented in Figure 4.3. There were no significant ($p \geq 0.503$) main effects observed in GPA between Prod and Sup qualities. Meanwhile, the main effect of Day 0 and Day 7 was studied by pooling results from all fractions and qualities. The GPA at Day 7 (0.12 ± 0.04 mg/g/h, n=17) was not found to be significant different ($p \geq 0.444$) from the GPA at Day 0 (1.5 ± 1.3 mg/g/h, n=15).

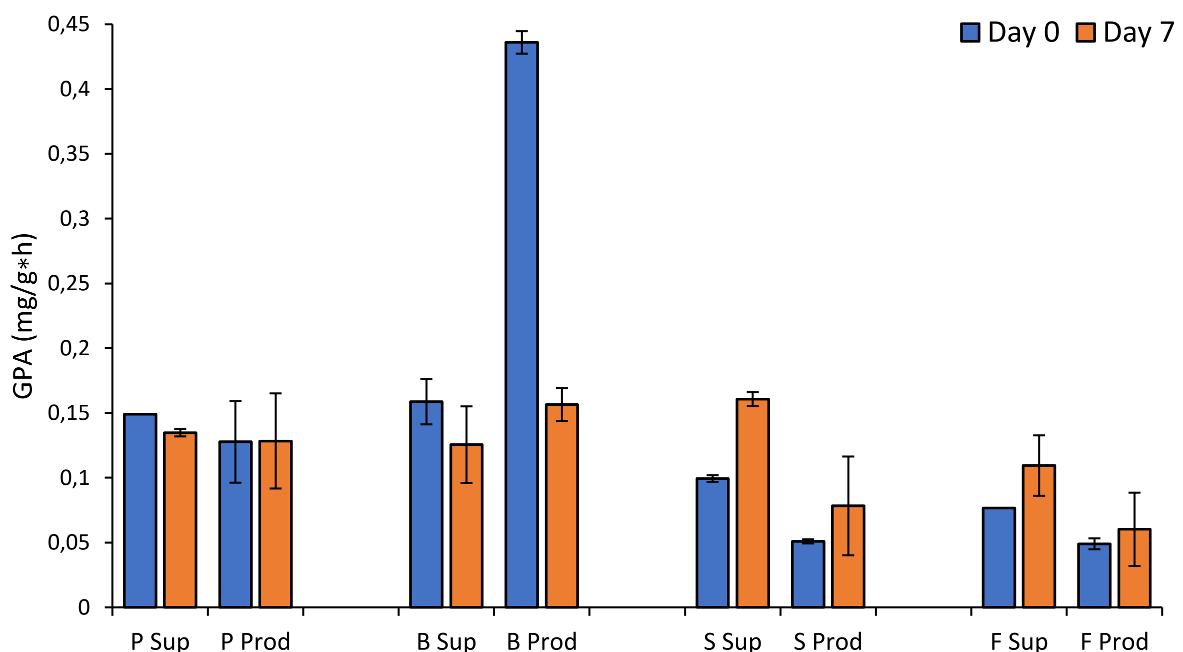


Figure 4.3. General proteolytic activity (GPA) in farmed Atlantic salmon. The results are sorted as Day 0 and Day 7, Superior (Sup) and Production (Prod) quality, and in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S), and Filet (F). All values are presented as trichloroacetic acid (TCA) soluble peptides liberated (mg) / wet weight (g) / hour and expressed as mean with SE as error bars (n=2-3) with the exception of P and F Sup day 0, where n=1.

Interestingly, the Prod Day 0 belly flaps had a GPA of 0.44 mg/g/h, which is significant higher ($p < 0.001$) than all the other fractions. The GPA was consistently high through all parallel measurements of the two Prod belly flap Day 0 enzyme extracts (Appendix 1, Table A-1) (Appendix 1, Table A-2). This sample causes the pooled GPA data for the belly flaps to be significantly higher ($p = 0.009$) than the GPA of the filets and deboned meat. Meanwhile, there were no significant differences ($p > 0.05$) between the Bits & Pieces and other fractions.

4.5. Acid Value

The results from the acid value analysis are presented in Figure 4.4. By studying the main effects of all Prod and Sup samples, there was found no significant difference ($p \geq 0.101$) in the acid values of the two qualities.

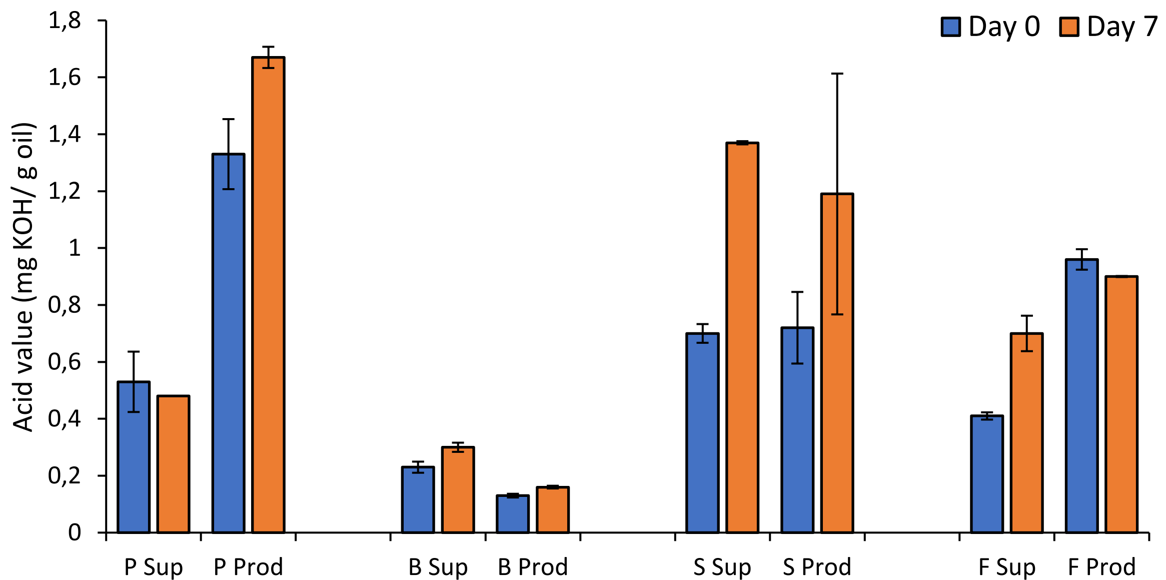


Figure 4.4. Acid value in oil extracted from farmed Atlantic salmon. The results are sorted as Day 0 and Day 7, Superior (Sup) and Production (Prod) quality, and in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S), and Filet (F). All values are presented as mg KOH/ g oil and expressed as mean with SE as error bars (n=2) with the exception of P Sup Day 7, where n=1.

The main effect between fractions was analysed by pooling the acid values from both days and qualities for each fraction. The acid value of the belly flap oil was found to be significantly lower ($p < 0.001$) than the other fractions. The low acid value indicates that the belly flap has less FFAs than the other fractions, both in fresh samples and after seven days of iced storage. It was found no significant differences between the other fractions.

There was found no significant difference ($p \geq 0.159$) in the main effect of acid values between Day 0 and Day 7. However, there seems to be a trend where the acid value is unchanged or increased from Day 0 to Day 7 in all samples.

4.6. ATP Degradation

The concentrations of IMP, Ino, and Hx are presented in Table 4-2, while the resulting Ki-value is presented in Figure 4.5. There was no significant main effect observed in the concentrations of IMP ($p \geq 0.230$), Ino ($p \geq 0.393$), and for the Ki-value ($p \geq 0.164$) between the Sup and Prod quality. However, the Sup quality average Hx concentration of 0.57 ± 0.07 µ mol/g (n=24) was found to be significantly higher ($p = 0.039$) than the Production qualities average Hx concentration of 0.40 ± 0.04 µ mol/g (n=24). Nonetheless, the difference in Hx concentrations was too small to make the H-value of the Sup quality (19.2 ± 3.0 %, n=24) significantly different ($p \geq 0.064$) from the H-value of the Prod quality (12.5 ± 9.2 %, n=24).

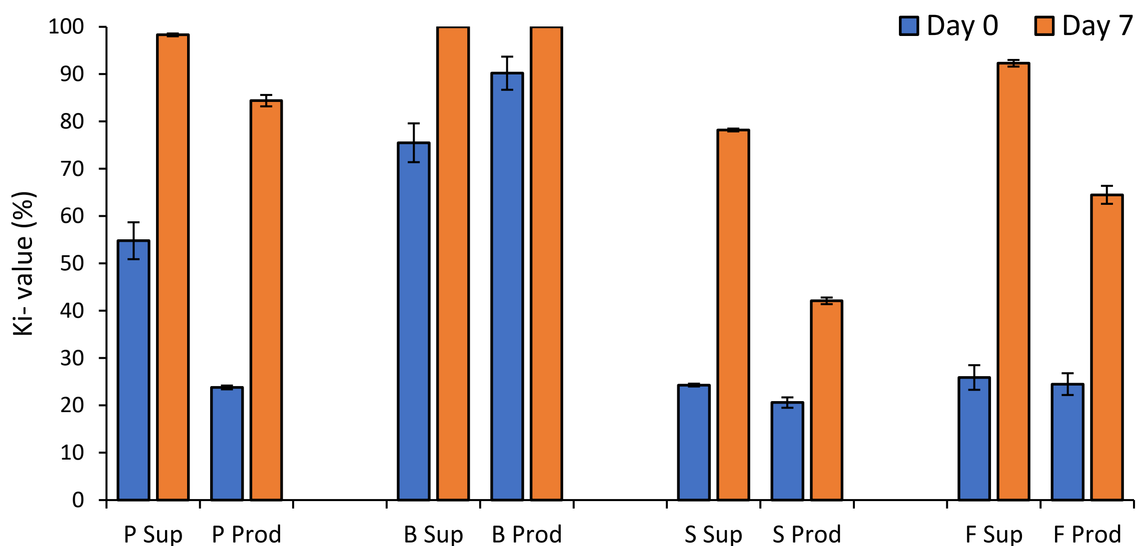


Figure 4.5. Ki- value calculated from the IMP, Ino and Hx values presented in Table 4-2. The results are sorted as Day 0 and Day 7, Superior (Sup) and Production (Prod) quality, and in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S) and Filet (F). All values are expressed as means with SE as error bars

The pooled Ki-values for both days show that the Ki-values significantly increase ($p < 0.001$) from Day 0 (42.4 ± 5.4 %, n=24) to Day 7 (82.5 ± 4.0 %, n=24). This is no surprise, as the Ki-value typically increases during iced storage due to the breakdown of IMP to Hx during autolysis. As they are presented in Figure 4.5, the Sup quality Bits & Pieces, and the belly flaps of both qualities, were found to have a significantly higher ($p < 0.001$) Ki-value compared to the other samples at Day 0. The high Ki-values are caused by their low concentrations of IMP at Day 0 (Table 4-2). Furthermore, these three fractions have Ki-values of 98-100% at Day 7, which is significantly higher ($p < 0.001$) than the other samples. Once again, the high Ki-values are caused by the low concentrations of IMP at Day 7.

The deboned meat has a low Ki-value at Day 0, which remains significantly lower ($p < 0.001$) than the other co-products Ki-values at Day 7. The Ki-values of the deboned meat Sup and Prod samples are even significantly lower ($p < 0.001$) than their filet Sup and Prod counterparts at Day 7. These findings suggest that the manually deboned meat might be more stable regarding conversion of IMP to Ino and Hx than other investigated fractions.

Finally, it was tested for correlations between fat content and ATP degradation rate due to patterns in the data. Using a Pearson correlation test, there was found a significant correlation ($r = 0.62$, $p = 0.006$) between the lipid content and Ki-values of the salmon co-products and filet. Furthermore, the combined concentration of IMP, Ino, and Hx are significantly higher ($p < 0.001$) in the filets (4.0 ± 0.2 μ mol/g, n = 12) than in the belly flaps (2.5 ± 0.1 μ mol/g, n = 12).

Table 4-2. Concentrations of IMP, Ino and Hx (µ mol/g) in all samples. The results are sorted as Superior and Production quality, in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S) and Filet (F), and as Day 0 and Day 7. All values are expressed as mean ± SE. The sample size (N) and p-value of an ANOVA analysis to test significant difference between fractions within each category are given. Different letters (a-f) within the same row indicate significant difference ($p < 0.05$) between fractions.

	Superior				Production					
	P	**B**	**S**	**F**	**P**	**B**	**S**	**F**	**p**	**N**
IMP (µ mol/g), Day 0	1.55 ± 0.16 ^{b}	0.59 ±0.07 ^{a,b}	4.37 ±0.52 ^{c}	3.48 ±0.32 ^{c}	3.75 ±0.14 ^{c}	0.26 ±0.10 ^{a}	3.77 ±0.07 ^{c}	3.52 ±0.27 ^{c}	< 0.001	3
IMP (µ mol/g), Day 7	0.04 ±0.01 ^{a,b}	0.00 ±0.00 ^{a}	0.81 ±0.03 ^{d}	0.24 ±0.02 ^{b}	0.49 ±0.02 ^{c}	0.00 ±0.00 ^{a}	2.47 ±0.10 ^{f}	1.30 ±0.08 ^{e}	< 0.001	3
Ino (µ mol/g), Day 0	0.54 ±0.09 ^{b,c}	1.36 ±0.02 ^{a,b,c}	1.15 ±0.19 ^{a,b,c}	1.00 ±0.22 ^{a,b,c}	0.94 ±0.03 ^{a,b}	1.61 ±0.16 ^{c}	0.78 ±0.03 ^{a}	0.96 ±0.06 ^{a,b}	0.002	3
Ino (µ mol/g), Day 7	1.90 ±0.45 ^{a,b,c}	1.29 ±0.07 ^{a}	2.21 ±0.10 ^{b,c}	2.33 ±0.05 ^{c}	2.16 ±0.09 ^{b,c}	1.89 ±0.09 ^{a,b,c}	1.45 ±0.05 ^{a,b}	1.96 ±0.09 ^{a,b,c}	0.008	3
Hx (µ mol/g), Day 0	0.32 ±0.02 ^{a,b}	0.51 ±0.21 ^{a,b}	0.25 ±0.00 ^{a,b}	0.23 ±0.04 ^{a,b}	0.23 ±0.02 ^{a,b}	0.59 ±0.02 ^{b}	0.19 ±0.03 ^{a}	0.17 ±0.01 ^{a}	0.013	3
Hx (µ mol/g), Day 7	0.75 ±0.06 ^{c}	1.22 ±0.17 ^{d}	0.70 ±0.01 ^{b,c}	0.59 ±0.01 ^{a,b,c}	0.51 ±0.02 ^{a,b,c}	0.74 ±0.04 ^{c}	0.34 ±0.02 ^{a}	0.41 ±0.01 ^{a,b}	< 0.001	3

IMP = Inosine monophosphate; Ino = Inosine; Hx = Hypoxanthine.

5. Discussion

The aim of this thesis was to study the chemical composition and stability of salmon co-products of Sup and Prod quality compared to filets. Knowledge about the proximate composition, protein solubility, FA profile, enzymatic activity, and ATP degradation is vital when investigating raw materials potentials and challenges as ingredients in foods. The information enables food producers to choose suitable ingredients for the foods' optimized functional properties and shelf life.

Østvik & Grimsmo (2010) found that industrial scale, mechanical production of salmon co-products require optimization before the co-products can be produced of consistent and high quality. Therefore, the quality of the manually produced co-products in this study could be regarded as the maximum achievable quality in industrial-scale production after an optimization phase. The quality of industrially produced co-products would also vary on the fileting grade of each production line. Example-wise, Bits & Pieces could contain high-quality trimmings from fileting as in this study, or only fatty trimmings with bones. Belly flaps are likely the easiest co-product to produce since most production lines today remove them during filleting.

One of the main findings of this study is that co-products from Sup and Prod quality salmon have similar chemical composition and stability. The presented results show no evidence that neither the co-products nor the filet of Prod quality salmon is less suited for human consumption than Sup quality salmon. This finding is important for the industry since Prod quality fish has to be processed in Norway to rectify any defects (NISF, 1999), which generates a lot of co-products. Meanwhile, most of the Sup quality co-products are exported as whole and gutted fish and processed in other countries. The possibility of using Prod quality co-products in foods might lead to new workplaces in the Norwegian industry and to a more sustainable utilisation of the available resources.

5.1. Proximate Composition

The Bits & Pieces and deboned meat co-products of this study have a similar protein content as the salmon filet (Figure 4.1). The high protein content makes these co-products excellent sources of protein. The filet protein content of 20.4% is similar to the 20.8% reported by Mæhre et al. (2018) but higher than the 18.8% and 18.6% reported by Atanasoff et al. (2013) and Aas et al. (2019), respectively. The differences in the reported protein content of the salmon filets could be caused by differences in feed, environment, or genetics (Shearer, 1994).

The Prod quality Bits & Pieces has a significantly lower ($p < 0.001$) lipid content than the Sup quality, from 10.3 to 18.9%. Salmon that are sorted as Prod quality have characteristics of sexual maturation, deformities, or handling defects (NISF, 1999). Deformities or handling defects would not predictably change the composition of salmon muscle. Meanwhile, sexual maturation is associated with increased water content and decreased lipid and protein content (Aksnes et al., 1986; Aunchalee et al., 2011). However, no significant differences ($p > 0.05$) have been found in the content of water, protein, or lipids in the pooled data for Prod and Sup quality salmon in the current study. The non-significant main effect indicates that the Bits & Pieces lipid content difference is not caused by actual differences between the Sup and Prod quality salmon.

The Bits & Pieces used in this study are sampled from the low-fat posterior end and the fattier anterior end of the salmon filet (Figure 3.3). The lipid content varies from 2-18 % in these parts of the filet (Katikou et al., 2001). The observed difference in fat and water content is likely caused by the inclusions of different cuts in the two batches. Furthermore, the high lipid content of the Sup fraction could be due to the inclusion of the fatty part of the salmon neck, which has a relatively high fat content (18.6%) (Olsen & Tobiassen, 2004).

The different lipid content in the two Bits & Pieces batches is interesting since it illustrates the variation within this co-product group. An industrial production line with a consistent filet trimming procedure would likely manage to produce Bits & Pieces batches with a stable fat content. However, the fat content could vary between production lines that trim the filets differently. The variation could be minimized by developing an industry standard for the production of Bits & Pieces, like Østvik et al. (2005) attempted to for deboned meat in 2005. An industry-standard should also develop a procedure for removing bones from the Bits & Pieces as this would increase their value.

In similarity with the manual deboned meat produced by Østvik et al. (2005) the manual deboned meat of this study had a lipid content close to 10%. The lipid content of industrial-produced deboned meat is expected to increase towards 23% as more soft, fatty tissue is

removed from the backbone. Furthermore, the inclusion of soft fatty tissue would decrease the otherwise high protein content in the deboned meat. Based on the tests performed by Østvik & Grimsmo (2010), the deboned meat is of the highest quality when the red meat of the backbone is removed without the fatty, soft tissue. However, this would decrease the amount of meat extracted from the backbone. A solution could be to remove meat from the frames twice, where only the red meat is removed the first time and then leftover meat the second time. While this would be a more laboursome process, it would also allow for all the meat to be extracted without compromising the high quality of the red meat left on the frames.

The belly flap deviates from the other co-products and the filet by having a significantly higher ($p < 0.001$) lipid content and a lower water and protein content than the other fractions (Figure 4.1). These results are not surprising as the belly flap serves as a fat storage for Salmon, while the other fractions are mostly muscle tissue. The high fat content makes the belly flap an excellent source of PUFAs. Furthermore, it could be used in fish mince products to increase their fat content, making them less dry. It is essential to be aware of the high fat content of the belly flap during product development as it would affect the functional properties of foods (Belton, 2000).

The fat content of belly flaps reported by the literature varies from 28 % (Aursand et al., 1994) to 44.7 % (Einen et al., 1998). The food and nutrition database "The FatSecret Platform API" reports that the belly flap of farmed salmon contains 36.3% fat and 12.6% protein (The FatSecret Platform API, 2020). Based on this, the lipid content of 34 % and protein content of 14 and 16 % observed in the belly flaps in this study are in agreement with the literature.

The ash content of all the co-products and the filet ranged from 0.9-1.5 % Figure 4.1. This is similar to the ash content of 1.0 % in salmon muscle reported by Atanasoff et al. (2013) and 1.1% in deboned meat reported by Østvik & Grimsmo (2010). The water and ash content were analysed twice in this study, with three parallels in each analysis. During analysis, some crucibles broke in the oven, and ash was blown out of the crucibles by the muffle furnace fan. These experimental errors resulted in the varying sample size for the water ($n = 5-6$) and ash ($n = 2-6$) content analysis.

For the proximate analysis, Day 7 samples were used instead of Day 0 samples primarily out of convenience under the assumption that the composition does not change during seven days of cooled (0-4 °C) storage. Technically, this assumption is wrong. The proximate composition of salmon changes slightly during iced storage due to drip loss, where water containing protein and lipids are released from the muscle. The lost protein is mainly WSP

(Rotabakk et al., 2018). Furthermore, Rotabakk et al. (2018) found that approximately 1% of the total wet weight of salmon filets is lost as drip loss during seven days of chilled storage. They analysed the drip loss composition and found that it consisted of approximately 86% water, 13.6% WSP, and 0.03% fat. Compared to the composition of the salmon fillets in this study (Figure 4.1), drip loss will result in a slight decrease in water content and a slight increase in the total protein, fat, and ash content. For further discussion in this study, we assume that the change in proximate composition due to drip loss is too small to interfere with the results. Furthermore, the one-day storage of the Prod quality salmon prior to processing are assumed to not affect its proximate composition (Figure 3.1).

5.2. Water- and Salt-soluble Protein

The WSP content of 8.2% found in the Sup and Prod quality filets of this study (Figure 4.2) is in agreement with previously reported results by Hultmann & Rustad (2004). Meanwhile, the filet SSP content of 2.5-2.7% is lower than the 8% reported by Hultmann & Rustad in the same study. The lower amounts of SSP are likely due to freeze denaturation of the salt extractable myofibrillar proteins in the frozen storage of the samples prior to protein extraction (Duun & Rustad, 2007; Mackie, 1993). Mackie *et al.* found that the freezing of cod at -14 °C in 30 weeks reduced the amount of extractable myofibrillar proteins by 100%. Dunn & Rustad found that the frozen storage of cod muscle at -40 °C in 43 days reduced the amount of SSP by 50%, while the amount of WSP was unchanged. The raw material used in this study was frozen in vacuum sealed bags at -80 °C for eight weeks before protein extraction.

Only the connective tissue (3-10% of total protein) is regarded as an insoluble protein fraction in fresh salmon samples (Mackie, 1993). The high content of rest, non-extractable protein in the samples (Figure 4.2) is likely caused by freeze denaturation of the myofibrillar SSP, which becomes insoluble upon denaturation. The freeze denaturation of myofibrillar proteins negatively affects both the texture and the functional properties of the fish (Mackie, 1993). The problem of freeze denaturation could be solved by processing the co-products while they are fresh. If the co-products must be frozen, Tolstorebrov et al. (2016) recommend that long-termed stored fish are stored at -35 °C in air-tight conditions.

Finally, the content of soluble proteins might have been affected by the seven days of storage (0-4 °C) before freezing. Hultmann & Rustad (2004) found that the content of WSP significantly decrease by approximately 1% from day 5 to day 14 of iced storage, where most of the decrease occurred between day 10 and 14. It is challenging to estimate how much the content of WSP decreases during the first seven days of storage based on this information, as the amount of WSP in the drip loss increase during time. Hultmann & Rustad (2004) found no significant decrease of SSP between day 5 and 14 of iced storage. For the purpose of this study,

we will assume that the content of WSP decreased between 0 and 1% while the content of SSP remained constant during the seven days of iced storage.

5.3. Fatty Acid Profile

The FA distribution of the filets analysed in this study (Table 4-1) is within the range specified in the FAO standard for farmed salmon (FAO, 2017). An exception is vaccenic acid (C18:1n-7) which is found in small concentrations (2.6-3.1%) in some samples of this study but reported as “not applicable” in the FAO standard. However, vaccenic acid does occur in the FAO standard for wild salmon.

The similar FA distribution in all fractions means that salmon co-products are an equally good source of n-3 PUFAS as the salmon filet. The amount of nutritional lipids such as DHA and EPA in foods based on salmon co-products mainly depend on the amount of fat in the co-products. It is of less importance if the lipid originates in the filet, belly flap, or deboned meat.

The uncertainty of measurement is unknown for the FA profile since the samples are analysed without experimental parallels. The measured FA distributions are assumed to be close to the true FA distributions as they are analysed GC, which is an established method known to yield results with high reproducibility. (Dorman et al., 2010). Furthermore, the salmon samples were stored seven days on ice before freezing, which might have affected the distribution of FAs. The salmon oil is rich in PUFAs, which is easily oxidized (Sohn & Ohshima, 2010). Lerfall et al. (2016) found that the percentage content of SFA and PUFA slightly increased during iced storage of salmon filets while MUFA slightly decreased. For the purpose of this study, we will assume that the distribution of FAs is unaffected by the seven days of iced storage, as the difference observed by Lerfall et al. (2016) was small. Moreover, any change in the FAs distribution is assumed to be similar in all samples as they were stored under identical conditions.

5.4. General Proteolytic Activity

The mean GPA of 0.07 mg/g/h found in the filet fraction () is similar to the findings of (Hultmann & Rustad, 2004) when incubating salmon filets at pH 25 °C. Meanwhile, the GPA of the samples in this study was lower than earlier reported Stoknes & Rustad (1995) when incubating salmon enzyme extracts at 60 °C with a pH 6 buffer. The currently observed activity in the filet is so low that it is difficult to detect, visible in the slight difference between incubated and not incubated samples (Appendix 1, Table A-1) (Appendix 1, Table A-2).

There was found no significant difference ($p \geq 0.503$) in GPA between Sup and Prod quality salmon. This finding is supported by Stoknes & Rustad, that found no observable difference in GPA level between superior and sexually mature salmon (Stoknes & Rustad, 1995). Their comparison between superior and sexually mature salmon can be transferable to this thesis' comparison between Sup and Prod quality salmon, as some of the Prod salmon are sexually mature. Furthermore, there was found no significant difference ($p \geq 0.444$) in the main effect of GPA between Day 0 and Day 7 samples. This is in agreement with Hultmann & Rustad that found no significant change in GPA during storage of salmon filets (Hultmann & Rustad, 2004). An observable change in proteolytic activity during storage would be interesting if the change were significantly different between fractions, as it would indicate different rates of proteolysis.

There was observed a significantly higher ($p = 0.009$) protease activity in the belly flap than in the filet, mainly caused by the high GPA in the Prod quality belly flap from Day 0. That sample had three times higher GPA than the other belly flap samples. As no other fractions exhibit similar variety in the GPA, it is cause to question whenever this result is possible to replicate in future studies. The measurements for the GPA analysis of this fraction (Appendix 1, Table A-2) have a low SE, indicating that possible irregularities must have happened before the protease extraction step, during storage or mincing of the sample. Alternatively, it might be possible that the proteases are almost inactivated in all samples except the Prod quality belly flap Day 0 sample. Inactive proteases would explain why the measured GPA is lower at 60 °C than earlier reported by Stoknes & Rustad (1995).

In previous studies on GPA, negative GPA values have been set to zero (Hultmann & Rustad, 2004; Stoknes & Rustad, 1995). The argument for doing this is that the amount of TCA soluble peptides can not be higher before incubation than after since the peptides are produced by the enzymatic cleavage of proteins. Therefore, negative GPA values must be caused by variations in the experimental method and should be excluded. However, negative GPA values have not been set to zero in the current study. The removal of negative GPA values before averaging creates a bias towards results with a higher GPA activity and a lower variation since the lowest measures of each parallel is set to zero while the highest measures are kept. To ensure reliable results, each parallel used in the result of this study derives from an independent protease extract that is measured in three parallels. Nontheless, the sample size of the GPA analysis do vary since outlying results caused by experimental error was excluded.

5.5. Acid Value

After seven days of storage (0-4 °C), all acid values are below the maximum acceptable level of 3 mg KOH/g recommended by FAO's standard for fish oils (Figure 4.4) (FAO, 2017). The FAO standard also recommends levels for the peroxide and anisidine values, which have not been measured in this study. The acid value is an indirect measure of the amount of FFAs in oil. Therefore, an increasing acid value during storage indicates lipase activity since lipases break down triglycerides into FFAs (Shahidi & Zhong, 2009).

The non-significant difference ($p \geq 0.159$) in the main effect analysis between Day 0 and Day 7 suggest that the lipase activity has been low during the seven days of storage in the present study. However, when comparing the acid value at Day 0 to Day 7 for each sample, there seems to be a trend where the acid values slightly increase (Figure 4.4). This is expected as the acid value normally increases during storage (Rodríguez et al., 2009). The acid value increases the most in the deboned meat and in the Prod quality Bits & Pieces, which might indicate that these co-products have higher lipase activity than the filets.

The acid values are only measured in duplicates, which provides a weak basis for statistics. Because of the small sample size, significant differences between samples might be lost (Morgan, 2017). Furthermore, the acid value provides limited information on its own. A full assessment of lipid oxidation should be performed to further study the state of lipid degradation in the salmon co-products. An analysis of primary oxidation products, such as peroxide value, and an analysis of secondary oxidation products, such as TBAR, should therefore be applied in further studies (Kumar et al., 2018).

5.6. ATP Degradation

Saito et al. (1959) recommend that fish with a Ki-value of 20% should be rated as fresh, 50% as medium fresh, and 70% as not fresh. According to Saito *et al.*'s categorization, samples from the filets, deboned meat, and Prod quality Bits & Pieces of this study are "fresh" at Day 0, while the Sup quality Bits & Pieces are "medium fresh" and the belly flaps are "not fresh" (Figure 4.5). The Day 0 belly flap's rating of "not fresh" indicates that Saito *et al.*'s scale based on K-values is a poor measure of the freshness of fatty salmon co-products.

After seven days of iced storage, all samples had significantly increased ($p < 0.001$) Ki-values, where most of the values were above 70%. The Ki-value development follows the apparent changes in eating quality of cod (Figure 2.6), where the major autolytic changes occur within six days *post-mortem*. During a storage experiment of sliced salmon filets stored at 1 °C, Sallam (2007) found that the K-value of filets was 18% at day 0, 55% at day 7, and 70% at day 15. The filet fraction of this study exhibits a somewhat faster increase in the Ki-value (Figure 4.5). The rapid increase in Ki-value might be due to the mincing of the salmon muscle, where biological membranes are broken, and air is mixed into the samples.

It was found significantly ($p=0.039$) higher Hx concentrations in the Sup quality salmon compared to the Prod quality (Table 4-2). This finding is unexpected since Sup quality salmon normally are regarded as a higher quality product than the Prod salmon, and increased Hx values are associated with an unpleasant bitter taste (Hong et al., 2017; NISF, 1999). Furthermore, the Prod quality "Day 0" salmon used in this study is one day older than the Sup quality "Day 0" salmon (Figure 3.1), which would usually result in an increased Hx concentration in the older Prod salmon (Sallam, 2007). The high concentrations of Hx in the Sup salmon might be caused by an inconsistent cold chain or contamination during processing, or by individual variation between salmon. Regardless, the different concentrations in Hx were too small to result in a significant difference ($p \geq 0.064$) in the H-values of the Prod and Sup qualities.

The belly flap fractions have an exceptionally high Ki-value, with an initial value of 80% and a Ki-value of 100% at Day 7 (Figure 4.5). The high Ki-values suggest that the belly flap fraction is especially susceptible to ATP degradation and should be handled accordingly. Meanwhile, the deboned meat exhibits a Ki-value significantly lower ($p < 0.001$) than even the filet. The low Ki-value is surprising as deboned meat could be contaminated with blood from the frames that contain endogenous enzymes (Falch et al., 2006). It is important to be aware of the differences between the manually deboned meat in this study (Figure 3.2) and industrial deboned meat. The manual deboning of meat results in a mince containing less blood and soft, fatty tissue than the industrial deboned meat, depending on the type of machine used

(Østvik et al., 2005). The low Ki-values found in the deboned meat in this study indicate that manually deboned meat is stable in regard to ATP degradation.

Interestingly, a correlation was found ($r = 0.62$, $p = 0.006$) between the lipid content and ATP degradation. The lean deboned meat and filets exhibits the lowest Ki-values, while the fatty belly flap and Sup quality Bits & Pieces co-products have the highest Ki-values. Furthermore, the total concentration of ATP-related compounds (IMP, Ino, and Hx) are significantly higher ($p < 0.001$) in the filet than in the fatty belly flap (Table 4-2). ATP is found in high concentrations in the mitochondria of muscle cells, which contain myofibrillar proteins (Mackie, 1993). Hence, the low concentrations of ATP-related compounds in the belly flap are likely caused by the significantly lower ($p = 0.007$) concentrations of myofibrillar SSP in the belly flap than in the other fractions (Figure 4.2). To summarize, these findings suggest that salmon fractions high in fat, or low in myofibrillar muscle tissue, have low concentrations of ATP-related compounds but a high ratio of Hx compared to IMP. This results in the high Ki-value observed in fatty salmon co-products.

6. Conclusion and Future Perspectives

The study aimed to investigate the chemical composition and stability of salmon co-products of Sup and Prod quality compared to filets. In this work, it has been found no evidence that co-products from Prod quality salmon are of poorer quality than co-products from the Sup quality equivalents. The co-products' proximate composition and protein solubility vary depending their original location on the fish, while the FAs are distributed similarly in all fractions. All co-products exhibited the same low acid value and GPA as the filet, except for a high GPA in the Prod quality Day 0 belly flap samples. The fatty belly flaps and Bits & Pieces where more susceptible to ATP degradation than the leaner deboned meat and the filet.

This study, in combination with Sletten (2020), contributes to the growing knowledge of salmon co-products as ingredients in foods. The preliminary results demonstrate that co-products from both Sup and Prod salmon are valuable raw materials with an overall acceptable quality compared to the filet. The composition of co-products will affect their functional properties in foods and should therefore be considered during product development. Salmon mince are susceptible to deterioration that must be processed in suitable conditions and frozen quickly thereafter. Lipid oxidation and freeze denaturation of SSP might be a problem during frozen storage. In the future, efforts should be made to increase the amount of salmon co-products used in food production to ensure the responsible and sustainable utilisation of available resources called for by the UNs 12th Sustainable Development Goal.

Stability of lipids and proteins during frozen storage is important to increase the utilisation of co-products from the Atlantic salmon industry. Therefore, in future studies, the stability of proteins and lipids in salmon co-products during frozen storage should be assessed. It would also be interesting to further optimize the GPA analysis to investigate whenever the high GPA observed in the belly flap is reproducible. The activity of specific proteases, such as cathepsins, trypsin, and collagenases, could also be studied. Moreover, further efforts to increase the use of salmon co-products in foods could benefit from collaborating with the processing industry. The industry has useful experience that might help with the development of industry standards to produce salmon co-products of a high and consistent quality. During product development, it would be insightful to experiment with different ingredients that might increase the stability, taste, or texture of the foods. Finally, developed products should be assessed by sensory analysis, and marketed as sustainable to increase acceptance on the market.

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Appendix 1 – Supplementary tables

Table A-1. General Proteolytic Activity (GPA) data for Superior quality salmon. The Bits & Pieces (P), belly flap (B), deboned meat (S) and Filet (F) fractions has been frozen fresh (Day 0) or stored at seven days (0-4 °C) before freezing (Day 7). Each protease extract are analysed in three parallels. The I-samples are incubated for an hour before the proteases are inactivated by 5% TCA, while the 0-samples are inactivated with 5% TCA prior to incubation. I- and 0-samples are presented as mg TCA soluble peptides/ g wet weight salmon. The GPA is expressed as the difference between the 1- and 0-samples, expressed as mg peptides liberated/ g wet weight salmon/ hour of incubation.

Quality	Day	Sample	Extract	Parallel	0-Samples (mg/ g)	I-Samples (mg/g)	GPA (mg/g/h)
Superior	Day 0	P	1	1	1.58	1.64	0.07
				2	1.45	1.73	0.28
				3	1.50	1.60	0.10
		B	1	1	1.63	1.31	-0.32
				2	0.91	1.36	0.46
				3	1.09	1.38	0.29
			2	1	1.26	1.57	0.31
				2	1.26	1.36	0.09
				3	1.16	1.29	0.13
		S	1	1	2.38	1.63	-0.75
				2	1.19	1.67	0.48
				3	1.17	1.75	0.58
			2	1	1.76	1.61	-0.15
				2	1.59	1.62	0.03
				3	1.69	1.52	-0.17
		F	1	1	1.60	1.54	-0.06
				2	1.56	1.52	-0.05
				3	1.08	1.41	0.33

	Day 7	P	1	1	1.44	1.50	0.06
				2	1.49	1.58	0.09
				3	1.37	1.62	0.24
			2	1	1.50	1.59	0.09
				2	1.51	1.66	0.15
				3	1.50	1.66	0.17
		B	1	1	1.27	1.44	0.18
				2	1.27	1.37	0.11
				3	1.29	1.41	0.12
			2	1	1.41	1.52	0.12
				2	1.43	1.45	0.02
				3	1.35	1.42	0.07
			3	1	1.48	1.71	0.22
				2	1.58	1.68	0.11
				3	1.59	1.78	0.19
		S	1	1	1.73	1.91	0.18
				2	1.72	1.81	0.08
				3	1.67	1.88	0.20
			2	1	1.80	1.99	0.19
				2	1.76	1.91	0.15
				3	1.71	1.86	0.15
		F	1	1	1.50	1.63	0.13
				2	1.54	1.60	0.06
				3	1.49	1.56	0.07
			2	1	1.53	1.68	0.14
				2	1.57	1.65	0.08
				3	1.53	1.70	0.17

Table A-2. General Proteolytic Activity (GPA) data for Production quality salmon. The Bits & Pieces (P), belly flap (B), deboned meat (S) and Filet (F) fractions has been frozen fresh (Day 0) or stored at seven days (0-4 °C) before freezing (Day 7). Each protease extract are analysed in three parallels. The I-samples are incubated for an hour before the proteases are inactivated by 5% TCA, while the 0-samples are inactivated with 5% TCA prior to incubation. I- and 0-samples are presented as mg TCA soluble peptides/ g wet weight salmon. The GPA is expressed as the difference between the 1- and 0-samples, expressed as mg peptides liberated/ g wet weight salmon/ hour of incubation.

Quality	Day	Sample	Extract	Parallel	0-Samples	I-Samples	GPA
Production	Day 0	P	1	1	1.51	1.55	0.04
				2	1.39	1.56	0.17
				3	1.49	1.49	0.00
			2	1	1.46	1.53	0.07
				2	1.41	1.58	0.17
				3	1.43	1.59	0.16
			3	1	1.57	1.78	0.21
				2	1.60	1.70	0.09
				3	1.61	1.86	0.24
		B	1	1	1.70	2.21	0.51
				2	1.74	2.16	0.42
				3	1.71	2.12	0.41
			2	1	1.78	2.21	0.44
				2	1.79	2.26	0.47
				3	1.79	2.17	0.38
		S	1	1	1.50	1.64	0.15
				2	1.53	1.52	-0.01
				3	1.52	1.54	0.02
			2	1	1.60	1.57	-0.02
				2	1.58	1.63	0.05
				3	1.53	1.65	0.13
		F	1	1	1.32	1.39	0.07
				2	1.35	1.42	0.08
				3	1.38	1.37	-0.01

			2	1	1.45	1.44	-0.01
			2	2	1.41	1.53	0.13
			2	3	1.49	1.53	0.05
	Day 7	**P**	1	1	1.81	1.89	0.07
	Day 7	**P**	1	2	1.85	1.96	0.11
	Day 7	**P**	1	3	1.86	1.96	0.10
	Day 7	**P**	2	1	1.77	2.00	0.23
	Day 7	**P**	2	2	1.90	2.05	0.15
	Day 7	**P**	2	3	1.88	2.00	0.12
	Day 7	**B**	1	1	1.80	1.91	0.11
	Day 7	**B**	1	2	1.78	1.94	0.16
	Day 7	**B**	1	3	1.82	1.98	0.17
	Day 7	**B**	2	1	1.88	1.97	0.08
	Day 7	**B**	2	2	1.79	2.05	0.26
	Day 7	**B**	2	3	1.78	1.94	0.17
	Day 7	**S**	1	1	2.05	2.15	0.10
	Day 7	**S**	1	2	2.04	2.19	0.14
	Day 7	**S**	1	3	2.01	2.11	0.11
	Day 7	**S**	2	1	2.16	2.15	-0.01
	Day 7	**S**	2	2	2.08	2.21	0.13
	Day 7	**S**	2	3	2.12	2.12	0.00
	Day 7	**F**	1	1	1.80	1.88	0.07
	Day 7	**F**	1	2	1.80	1.94	0.13
	Day 7	**F**	1	3	1.83	1.89	0.06
	Day 7	**F**	2	1	1.86	1.89	0.04
	Day 7	**F**	2	2	1.93	1.89	-0.04
	Day 7	**F**	2	3	1.82	1.92	0.10

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