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Veronica Hjellnes

"It's not uncomplicated to say you simply cannot do it like this anymore" – a study of the Norwegian whitefish industry and the potential for improved utilization of rest raw materials

NTNU
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Acknowledgement

This thesis presents the memoirs from my voyage of discovery in the whitefish industry, including the experimental work carried out at the Department of Biotechnology and Food Science, NTNU.

Most of all I would like to thank my supervisors Associate Professor Eva Falch and Professor Turid Rustad. You have inspired, corrected, and guided, while giving me the freedom and confidence to further develop my ideas and skills as a researcher. Thanks to you this thesis has become something I am proud to present. I would also like to thank my colleagues and fellow phD-students, especially Sherry and Anna for being my friends ever since we met 3 years ago.

I have had the joy of supervising several bachelor and master students over the last years, and a special thanks to Stine and Elin for an invaluable effort in the laboratory. And Associate Professor Ida-Johanne Jensen, it was a pleasure discussing, sharing ideas, and learning from you.

I am grateful to my loving parents who taught me the value of hard work, and to Oskar, who showed me that there is more to life than work as well. You and Boris fill my life with love, laughter, and happiness. And to my little sister, my heart, who inspire me every day. I could never have done this without you.

At last, I would like to thank all those who opened the door when I came knocking and answered with a friendly voice when I called. To the participants of the case study, who met me with such openness and invited me into their world: thank you. Besides everything else, I felt trusted. These experiences are more valuable than any academic achievement will ever be.

Veronica Hjellnes
Trondheim, October 2021

Summary

The Norwegian whitefish industry has long traditions and constitutes an important part of both economy and food production. Whitefish is a collective term for several lean fish species, the most important being cod (*Gadus morhua*) and saithe (*Pollachius virens*). Processing of whitefish generates substantial amount of rest raw materials (RRM) that are mainly used for animal feed, biofuels or wasted. However, in order to meet the growing demand for nutrition, while simultaneously protecting our environment, we need to change the way we produce food. Whitefish RRM is a source of several nutritional components, including high-quality proteins, that can be used for human consumption as part of a sustainable food production.

The aim of this thesis was to present a systemic and innovative approach to the Norwegian whitefish industry and the objective of improving the utilization of whitefish RRM generated during catch, landing, and processing. This approach involved the use of two paths of obtaining knowledge, and the combinations of those, to get a thorough understanding of the current potential for improved utilization of whitefish RRM. The first path involved the use of laboratory experiments to evaluate the effect of spawning on saithe RRM, the potential for upscaling biotechnological processing for bulk production of protein products and the refinement of these to increase bioactive properties. The second path involved a case study within the Norwegian whitefish industry to investigate how experiences, attitudes and practices among fishers, and the circumstances affecting these, could enable or complicate efforts to improve utilization of RRM. The knowledge obtained from the laboratory experiments and the case study were then combined in an overall evaluation.

Saithe RRM mainly consist of heads, backbones, and viscera. Spawning did not affect the nutritional composition of heads and backbones. These RRMs are also relatively stable compared to viscera and were selected for further processing. Processing of saithe RRM involved mincing, enzymatic hydrolysis and membrane ultrafiltration. Enzymatic hydrolysis in bioreactors enabled extraction of RRM protein content to a high-quality saithe protein hydrolysate (SPH). Regarding processing equipment, a need for powerful and energy-efficient solutions for mincing, agitation, and dewatering were identified. SPH was further processed by membrane ultrafiltration to concentrate small peptides, which are associated with several health beneficial bioactive properties including the ability to work as antioxidants. Spawning neither affected the quantity and quality, nor the antioxidative activity of SPH. Membrane ultrafiltration enabled a concentration of small peptides but did not increase antioxidative

activity compared to SPH. Significant amount of the RRM protein content ends up in the secondary products of processing, which makes it important to find areas of applications for these as well.

Eight interviews with fishers of the whitefish industry resulted in the creation of three main themes. These concerned the term sustainability and its interpretation, the fragmented organization of the value chain, the development and implementation of regulations, and how this can affect rationalization, behaviour, and attitudes among fishers.

This thesis has identified logistical, technological, and sociocultural factors, in addition to factors concerning the raw material itself, that could affect the potential for improved utilization of whitefish RRM. While the availability and seasonal stability of saithe RRM could positively affect this potential, insufficient processing solutions, communication, management, and organization of the value chain could have a negative effect. This work does not provide a final solution, nor an all-encompassing truth, but can inspire other natural scientists to look beyond the limitation of traditional research methods and see the value in adopting and developing new methods for obtaining knowledge. Knowledge that could contribute to a sustainable development within the Norwegian whitefish industry, but also on a global level.

Terminology

DH	**D**egree of **H**ydrolysis
EFSA	**E**uropean **F**ood **S**afety **A**uthority
EU	**E**uropean **U**nion
FAO	**F**ood and **A**griculture **O**rganization of the United Nations
GHG	**G**reenhouse **G**as
MWCO	**M**olecular **W**eight **C**ut-**O**ff
NSC	**N**orwegian **S**eafood **C**ouncil
PER	**P**rotein **E**fficiency **R**atio
ROS	**R**adical **O**xygen **S**pecies
RRM	**R**est **R**aw **M**aterial
SPH	**S**aithe **P**rotein **H**ydrolysate
SDG	**S**ustainable **D**evelopmental **G**oal
UF	Membrane **U**ltra**f**iltration
UN	**U**nited **N**ations

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Aims and scope

The aim of this thesis is to present a systemic and innovative approach to the Norwegian whitefish industry and the objective of improving the utilization of whitefish rest raw material (RRM). My search for factors that could affect the potential for improved utilization of RRM has involved a combination of different methods to understand the complexity of this objective. It is my opinion that albeit providing a safe and reliable frame for conducting research, the traditional methodology and practice of natural science can hinder a creative process and the development of solutions to fit complex problems. The work of this thesis thus aims to combine the knowledge obtained from laboratory experiments with that obtained from observations and interviews conducted within the whitefish industry.

The laboratory experiments were conducted to evaluate the effect of spawning on saithe RRM, the potential for upscaling biotechnological processing for bulk production of protein products and the refinement of these to increase bioactive properties. All experiments were conducted with a continuous focus on the adaptability to industrial processing lines, and the main findings forms the first part of the results. The second part of the results is the analysis of a case study consisting of eight interviews with fishers of the coastal fleet. The numerous formal and informal encounters with stakeholders of the whitefish industry have also contributed to the reflections and perspectives presented throughout this thesis. An attempt to combine the insight obtained from the laboratory, observations and interviews forms the third and final part of the results.

This thesis builds on three papers specified on the following page. While Paper I introduce the Norwegian whitefish industry and the basis for the chosen approach, Paper II and Paper III presents the results of the laboratory experiments on protein recovery and refinement respectively. Reference is made to the papers throughout this thesis.

As it is important for me that the content of this thesis reach as many as possible, I have attempted to use a language that is not exclusive for the academic sphere. It is my sincere wish that this thesis can contribute to ease the strict framework of research and development, and to increase consideration, collaboration, and exchange of knowledge across disciplines. The aim of this work was never to provide a final solution, nor an all-encompassing truth, but to inspire other natural scientists to look beyond the limitation of traditional research methods and see the value in adopting and developing new methods for obtaining knowledge. Knowledge that could contribute to a sustainable development within the Norwegian whitefish industry, but also on a global level.

List of papers

I. Hjellnes, V., Rustad, T., & Falch, E. (2020). The value chain of the white fish industry in Norway: History, current status and possibilities for improvement – A review. *Regional Studies in Marine Science*, *36*. doi:10.1016/j.rsma.2020.101293.

II. Hjellnes, V., Rustad, T., & Falch, E. (2021). Enzymatic hydrolysis of pre-spawned and spawning saithe (*Pollachius virens*) in bioreactors and its potential for implementation in the Norwegian whitefish industry. *Manuscript submitted for publication.*

III. Hjellnes, V., Rustad, T., Jensen, I. J., Eiken, E., Pettersen, S. M. & Falch, E. (2021). Ultrafiltration of saithe protein hydrolysates and its effect on antioxidative activity. *Catalysts, 11*(9). doi:10.3390/catal11091053.

1 Food

Food is inevitably the most important element of our survival. We need food to live, to grow and ultimately to perform the activities we desire. Food is a source of pleasure and comfort, but also pain and agony when the resources are limited. A distressing number of 690 million people, or 8.9% of the world's population, is undernourished and 3 billion people cannot afford a healthy diet consisting of diverse food groups, adequate calories and nutrients (FAO, IFAD, UNICEF, WFP, & WHO, 2020). FAO (2017) estimates that the worlds food production must rise by 50% to meet the demands of the growing population by 2050. The need for more high-quality protein is further amplified by the population`s increasing wealth and awareness around nutrition. Increasing food production will put an increased strain on our natural resources, but also our environment, as the way we produce and consume food today is responsible for 21-37% of the greenhouse gas (GHG) emissions resulting from human activity (FAO et al., 2020). Improving the utilization of food resources and reducing food losses in quantity and quality along the food value chains (SDG 12), increasing the availability and reducing the cost of healthy food (SDG 2) and reducing the GHG emission (SDG 13) will be of crucial importance to meet the UN 2030 Agenda for Sustainable Development and the interconnected Sustainable Development Goals (SDGs). As important contributors to food production and providers of food resources rich in high-quality protein, improving the practice of fish industries on a global level is highly relevant for sustainable development.

2 The Norwegian whitefish industry

The whitefish industry has long traditions in Norway, where fishers have harvested the benefits of the resource-rich elongated coastline since the stone age. A highly organized and complex industry gradually emerged from the small fishing communities, where people primarily consumed their own catch. The productive and continually growing fish industry has consequently become a very important part of Norwegian food production and economy, where aquaculture of Atlantic salmon (*Salmo salar*), wild capture of pelagic fish and whitefish are the main contributors. Whitefish is a collective term for the species cod (*Gadus morhua*), saithe (*Pollachius virens*), haddock (*Melanogrammus aeglefinus*), ling (*Molva molva*), and tusk (*Brosme brosme*). Fish is a well-documented source of several health beneficial nutritional components like proteins, omega-3 fatty acids, vitamins, and minerals. Fish contributes to around 18% of the total animal protein consumed globally, and sustainable fisheries are thus crucial to prevent climate change and reduce hunger and malnutrition (FAO, 2017).

The Norwegian whitefish industry consist of a coastal and a seagoing fleet, that in total captured 650 000 tons whitefish with a value of 10.9 billion NOK in 2020 (Fiskeridirektoratet, 2021; Nøstvold, Svorken, Ødegård, Andersen, & Young, 2019). Cod and saithe constituted 50% and 30% of the capture weight respectively. While 70% of the capture value came from cod, saithe only accounted for 16%. Saithe therefore positions itself as a less income generating product for the fishers compared to cod, which might have implications for handling and processing.

Fisheries account for 55% by volume and 30% by value of the total amount of exported seafood in Norway (Hatlem, 2021). This included export of 410 000 tons of whitefish with a value of 14.3 billion NOK in 2020, of which 68% was cod and 17% saithe (NSC, 2021). An overview of the whitefish export distributed among the various groups of products are presented in Table 1. Frozen fish constituted the majority (45%) of the export, while 24% was exported as fresh product. The traditional whitefish products "tørrfisk" (stockfish), "klippfisk" (clipfish) and "saltfisk" (saltfish), made from processing by drying and salting, constituted 28% of the export. A small fraction of 2.8% of the export consisted of niche products like dried, smoked, or salted heads, roe (caviar), liver etc., while 0.1% consisted of prepared or preserved products like surimi.

Table 1: *Norwegian export of whitefish in 2020 distributed among the various groups of products, associated amounts (tons, %) and value (billion NOK, %). Numbers are obtained from NSC (2021).*

Exported product	Amount (tons)	Amount (% of total)	Value (billion NOK)	Value (% of total)
Fresh fish	99 270	24.3	3.03	21.2
Frozen fish	184 651	45.2	5.22	36.5
Dried/salted fish	112 751	27.6	5.76	40.3
Dried/smoked/salted heads, roe, liver etc.	11 439	2.8	0.24	1.7
Prepared/preserved fish	409	0.1	0.03	0.2

Based on these numbers, the value per ton of fish increases substantially from landing to export, where export of processed products yields a higher value than export of unprocessed fish. Norway exports approximately 90% of whitefish as gutted and deheaded whole fish (NSC, 2021). This leaves a tremendous potential for value creation and employment by moving more of the processing to Norway. While the yearly catches have increased, merely one tenth the number of people are registered as fishers today compared to the mid-20th century (SSB, 2021). Industrial practice has also changed, from many small scale fishers to the large vessels of the seagoing fleet with a high level of efficiency. Increasing the domestic processing of fish will thus require innovation, a high degree of digitalization and automation in line with the Industry 4.0 mindset (Lasi, Fettke, Kemper, Feld, & Hoffmann, 2014).

The way we produce food is crucial to achieve a sustainable development, as food production can have adverse effects on both the climate, the environment and our health (Halloran et al., 2020). Several Norwegian laws and regulations have been implemented with the purpose of maintaining a sustainable management of marine resources. Wild capture of marine species is controlled through the Regulation on the Practice of Fishing in the Sea (Lovdata, 2005), where §48 describes a requirement to bring all catch ashore, commonly referred to as the landing duty. Further, the Regulation on Landing and Closing Notes (Lovdata, 2015) ensures that all relevant data is registered upon landing of the catch.

3 Rest raw materials

Processing of natural resources to produce food for human consumption inevitably generates by-products, that themselves can be nutritious. The term by-product is however defined as production leftovers that are not intended for human consumption (EU, 2009). In this thesis rest raw material (RRM) is used to describe those leftovers that can be used for food purposes when handled according to the regulatory framework for food production (Lovdata, 2009). Fish RRM generally consist of everything that is left after production of the filet, which is the main product but can account for merely one third of the fish weight (Falch, Rustad, & Aursand, 2006; Falch, Rustad, Jonsdottir, et al., 2006). During the peak spawning season, niche products like liver and roe are also high-value products. However, fish heads, backbones and viscera generally constitute the RRM. Similar to fish derived food products, RRM can be a source of several valuable nutritional components including high-quality protein and lipids (Ghaly, Ramakrishnan, Brooks, Budge, & Dave, 2013).

RRM is generated throughout the whitefish value chain, which consist of all the consecutive steps from the fish is captured until it ends on our dinner plate (Kaplinsky & Morris, 2000). As discussed in Paper I, the seagoing and costal fleet constitutes the first step of the chain, while landing, processing, distribution, and consumption generally follow in a linear fashion. This thesis deals with the RRM that is generated during the first three steps of the value chain, as presented in Figure 1.

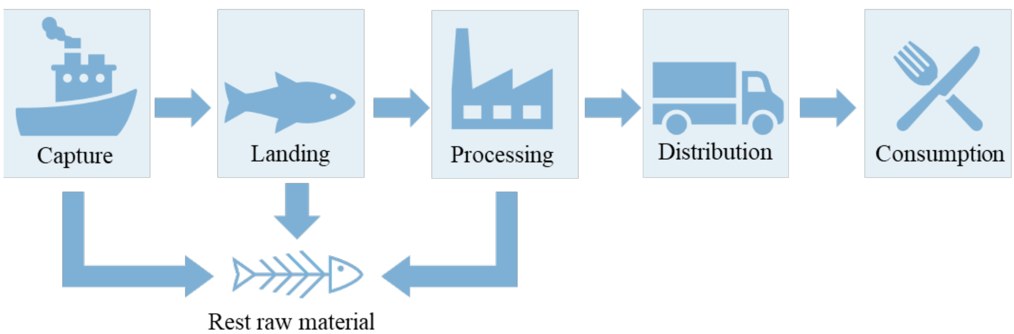

Figure 1: *The whitefish value chain consists of capture, landing, processing, distribution, and consumption. This thesis deals with the rest raw material that is generated during the first three steps.*

The Norwegian whitefish industry generates approximately 300 000 tons of RRM each year, of which 60% is utilized mainly for production of low-value products like animal feed and biofuel (Myhre, Richardsen, Nystøyl, & Strandheim, 2020). The remaining 40% is wasted and make up the majority of wasted RRM from marine sources. Paper I discuss the reasons for the low degree of RRM utilization in the whitefish industry compared to aquaculture, one of the most important being the logistical challenges associated with storage and transport. Fish RRM is highly perishable and deteriorates rapidly if not processed quickly after capture, refrigerated, or preserved by other means. Heads and backbones are however relatively stable RRMs compared to viscera that has a higher content of microorganisms, endogenous enzymes, and lipids prone to oxidation (Rustad, Storrø, & Šližytė, 2011). As discussed in Paper II, head, backbone, and viscera comprise about 15-20%, 15% and 10-15% of the fish weight respectively (Aspmo, Horn, & Eijsink, 2005; Falch, Rustad, & Aursand, 2006; Ghaly et al., 2013; Gildberg, Arnesen, & Carlehög, 2002). The major fractions of RRM generated are however reported to be head and viscera, while backbones constitutes a smaller part (Myhre et al., 2020). This is a direct result of the degree of processing currently taking place in Norway. As mentioned in Section 2, 90% of the whitefish is exported as gutted and deheaded whole fish and only 3 vessels of the seagoing fleet is currently involved in filet production (Myhre et al., 2020). This means that significant amounts of RRM is being generated in the countries where the processing takes place.

Sustainability is a broad and complex term with continuously increasing popularity and leverage in the social debate. In 1987, the UN Brundtland Commission defined sustainability as *meeting the needs of the present without compromising the ability of future generations to meet their needs*, and sustainable development has since become a multidimensional concept, encompassing both environmental, economic and social aspects (Halloran et al., 2020; UN, 2015). Improving the utilization of RRM is an important part of sustainable development, as it contributes to a more sustainable use of natural resources, a more sustainable food production and a circular economy (Jurgilevich et al., 2016). A question then arises of what is needed to find the best solutions to ensure that the utilization of whitefish RRM is improved.

4 Bioactive peptides and antioxidative activity

Fish RRM can be a source of bioactive peptides. These are inactive parts of native proteins, but can be released through biotechnological processing (Kim & Mendis, 2006). Bioactive peptides are small molecules of 3 - 20 amino acids with inherent health-beneficial properties beyond being a source of nutritious amino acids (Gao et al., 2021; Wijesekara & Kim, 2010). The wide range of potential bioactive properties of peptides includes their ability to work as antioxidants in pharmaceuticals, nutraceuticals and food (Hartmann & Meisel, 2007). Bioactive peptides and their potential health benefits have also been discussed in Paper I and Paper III.

Antioxidants are molecules with the ability to prevent undesirable oxidative reactions by neutralizing potentially harmful substances (Gulcin, 2020). Oxidative reactions are caused by reactive oxygen species (ROS) generated through the process of converting sugars to energy in our body and through lipid oxidation in food. Excessive levels of ROS is associated with several diseases as well as reduction of food quality due to rancidification (Pihlanto, 2006; Sarmadi & Ismail, 2010). The undesirable activity of ROS can be neutralized by antioxidants that are naturally occurring in humans, animals, and plants, or added to food products as natural or synthetic additives. α-Tocopherol (Vitamin E), ascorbic acid (Vitamin C) and plant polyphenols are examples of naturally occurring antioxidants, while synthetic antioxidants include butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Gulcin, 2020; Halliwell, 1996). Natural processes in our body and processing, production, and storage of food regularly cause excessive levels of ROS, which creates a high demand for safe and efficient antioxidants. Peptides from both cod and saithe have been found to exhibit antioxidative activity in vitro (Farvin et al., 2016; Girgih et al., 2015; Jensen, Abrahamsen, Maehre, & Elvevoll, 2009).

Antioxidants work by several mechanism as discussed in Paper III, including neutralization of ROS and binding of metals that can otherwise promote ROS formation (Gulcin, 2020; Huang, Ou, & Prior, 2005). As a result of this there are also a wide range of methods for analysing antioxidative activity. The individual methods analyse one specific mechanism under a specific set of conditions, and it is thus necessary to combine several methods to evaluate a potential antioxidant. The complexity of evaluating bioactivity is further illustrated by the imbalance and discrepancy occurring between *in vitro* and *in vivo* studies (Jensen & Mæhre, 2016; Zamora-Sillero, Gharsallaoui, & Prentice, 2018). While laboratory *in vitro* methods can be effective for evaluating antioxidative activity, it is important to acknowledge that the obtained results cannot be extrapolated to the stability and availability in food products or biological systems like humans. As part of a food matrix, bioactive peptides must remain intact without interacting with other food components that could reduce their bioactivity (Udenigwe & Fogliano, 2017). When ingested, bioactive peptides are further exposed to the hydrolytic activity of digestive enzymes in the gastrointestinal system. Digestion would in turn prevent the intact peptide from being absorbed through the gut lining and transported through the bloodstream, a requirement for it to exert its antioxidative activity in humans (Hernández-Ledesma, del Mar Contreras, & Recio, 2011).

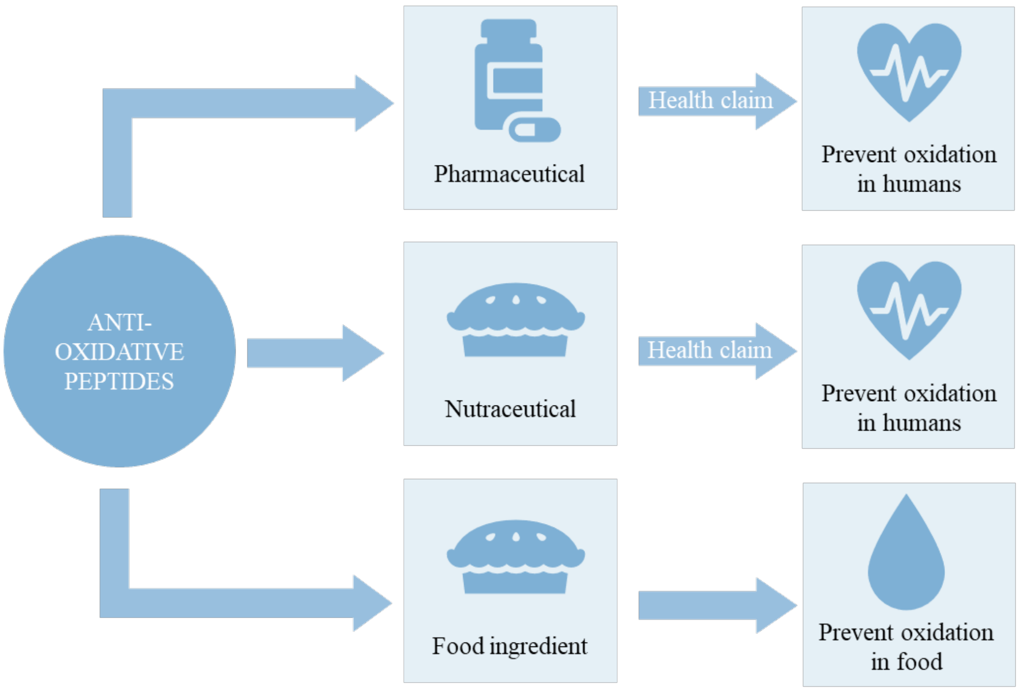

Figure 2: *Antioxidative peptides can be used as pharmaceuticals or as part of nutraceuticals to prevent oxidation in humans. This would require documentation to substantiate a health claim approval by local regulatory authorities. A third option is to use antioxidative peptides as a food ingredient to prevent oxidation in food.*

Figure 2 presents three possible ways of using antioxidative peptides in products for human consumption. Antioxidative peptides can be used as a pharmaceutical, or as part of a nutraceutical, to prevent oxidation in humans. The third option is use as a food ingredient to prevent oxidation in food. Regarding the use of bioactive peptides in products for human consumption, it is important to consider the restriction and requirements formulated in the Regulation on Nutrition and Health Claims in Foodstuffs (Lovdata, 2010). A health claim is defined as any claim that suggests a link between a food or its constituents and health, as would be the case for marketing antioxidative peptides as a pharmaceutical or a nutraceutical (Figure 2). Health claims are strictly regulated and comprehensive scientific documentation, including clinical studies, is required for approval. No health claims on bioactive peptides derived from fish has so far been approved in the European Union (EU) and consequently not in Norway. However, several peptide products have been approved as safe for human consumption by the European Food Safety Authority (EFSA) and are available on markets outside EU (Chalamaiah, Ulug, Hong, & Wu, 2019; EU, 2015). These include Valtyron® and PreCardix® that are both products claiming to contain blood pressure reducing peptides, from sardine (*Sardinops sagax*) and northern shrimp (*Pandalus borealis*) shell respectively (Marealis, 2021; Uksnøy, 2019). PreCardix® is currently marketed in Canada and the United States.

5 Enzymatic hydrolysis

To enable the use of fish RRM for human consumption, proteins and other valuable components must be extracted through some type of processing. Several biotechnological and chemical processing methods have been applied to fish RRM, including silage, chemical and enzymatic hydrolysis (Aspevik et al., 2017). Fish silage is a common way to utilize RRM in Norway, and is widely used as a lipid and protein source in fish and animal feed (Rustad, 2003). However, both silage and chemical hydrolysis involve the use of organic acids and other potentially harmful substances that limits the use of the extracted proteins in food. Enzymatic hydrolysis constitutes a milder processing alternative, where the activity of enzymes is used to cleave RRM protein to water-soluble peptides (Kristinsson & Rasco, 2000). The peptides are extracted in the main processing product called a protein hydrolysate, which subsequently can be dried to obtain a protein powder. Fish oil (lipid) is also extracted during the enzymatic hydrolysis, while the remaining components form an insoluble sludge fraction. All three processing products contain valuable nutritional components as shown in Figure 3.

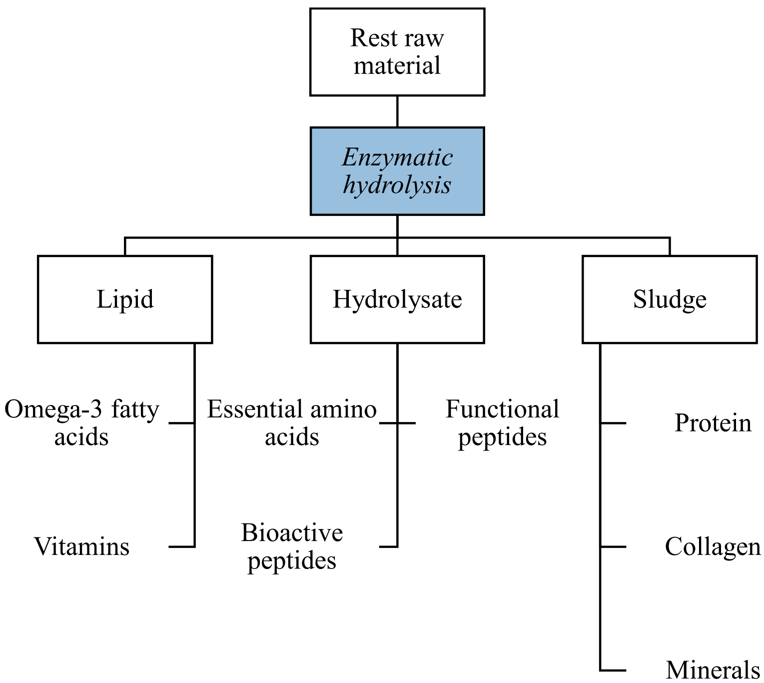

Figure 3: *Enzymatic hydrolysis enables separation of the valuable nutritional components of fish rest raw material into a lipid, water-soluble (hydrolysate), and insoluble (sludge) fraction.*

The enzymatic activity can originate from enzymes already present in RRM (endogenous) or added commercial enzymes. Enzymes that are commonly used to produce ingredients for food are Alcalase®, Flavorzyme®, and Protamex® of microbial origin, trypsin and pepsin of animal origin and papain and bromelain of plant origin (Zamora-Sillero et al., 2018). The choice of enzyme, as well as other processing parameters like temperature, time and pH, will influence the properties of the processing products. Papain and bromelain, either separate or in combination, has been shown to effectively hydrolyse fish RRM (Fan et al., 2019; Hou, Li, Zhao, Zhang, & Li, 2011) and was consequently used for the laboratory experiments presented in this thesis. A selection of studies conducted on enzymatic hydrolysis of fish RRM with the use of reasonable and industrially relevant processing conditions is presented in Table 2.

Table 2: *A selection of studies conducted on enzymatic hydrolysis of fish rest raw material with the use of reasonable and industrially relevant processing conditions.*

Raw material	Temperature	Enzymes	Time (minutes)	Scale	Reference
Atlantic cod (*Gadus morhua*) backbone	55°C	0.1% Protamex™	60	Lab	Šližytė et al. (2009)
Herring (*Clupea harengus*) head, backbone, skin, and viscera	55°C	0.1% Papain + Bromelain 0.1% Alcalase®	60	Pilot	Šližytė, Carvajal, Mozuraityte, Aursand, and Storrø (2014)
Atlantic salmon (*Salmo salar*) head, backbone, and viscera	52°C	0.1% Papain + Bromelain	120	Lab/ Pilot	Opheim et al. (2015)
Threadfin beam (*Nemipterus japonicus*) backbone	50°C	<0.7% Papain <3.8% Bromelain	60	Lab	Gajanan, Elavarasan, and Shamasundar (2016)
Fishmeal produced from cod (*Gadus morhua*) and saithe (*Pollachius virens*) head and viscera	55°C	0.1% Protamex™	60	Lab	Ween, Stangeland, Fylling, and Aas (2017)
Atlantic cod (*Gadus morhua*) head	50°C	0.1% Papain + Bromelain	60	Pilot	Remme and Austnes (2020)
Rainbow trout (*Oncorhynchus mykiss*) head	50°C	0.1% Papain + Bromelain	60	Lab	Kvangarsnes, Kendler, Rustad, and Aas (2021)

The experiments conducted in the studies presented in Table 2 are either laboratory (lab) scale or pilot scale, which has been the standard for most studies on enzymatic hydrolysis of fish RRM (Gao et al., 2021). In this thesis, lab scale is used to describe hydrolysis experiments that use simplified equipment setup to mimic an industrial process, whereas pilot scale describes hydrolysis experiments that use equipment that are more or less identical to an industrial process for the purpose of test-running. It has been common practice to advance directly to pilot scale, which in many cases has demonstrated that results obtained from lab scale experiments does not necessarily correspond to those obtained in an industrial setting. As discussed in Paper II, bioreactors can in this context be used as a step in a controlled upscaling from lab scale to pilot and industrial scale (Figure 4). By increasing the possibility for control and monitoring of the enzymatic hydrolysis, bioreactors can adapt and improve processing to facilitate a successful technology transfer to the industry.

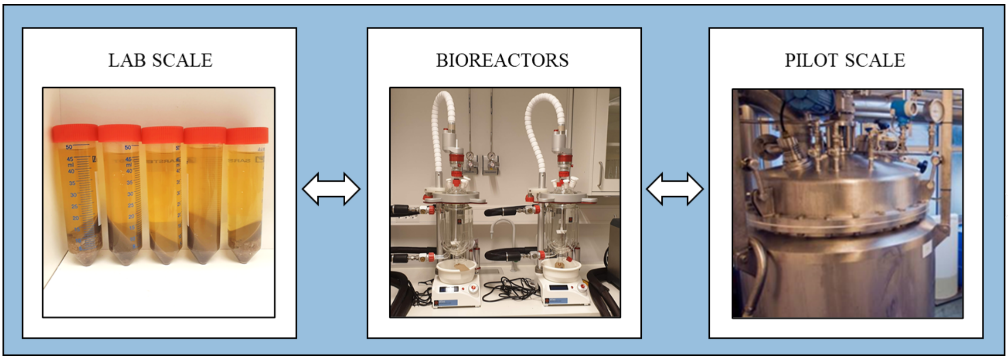

Figure 4: *Bioreactors (middle) can be used as a step in a controlled upscaling from laboratory scale (left) to pilot and industrial scale (right).*

6 Membrane ultrafiltration

As discussed in Section 4, Paper I and Paper III, fish RRM can be a source of bioactive peptides which can be released through processing by enzymatic hydrolysis. However, the resulting protein hydrolysate is a mixture of peptides with various molecular weights and functional properties. Membrane ultrafiltration (UF) is a processing and refinement method that can be used to concentrate small peptides in the protein hydrolysate to increase the bioactivity of the obtained product (Gao et al., 2021; Kaur, Sharma, Jaimni, Kehinde, & Kaur, 2020; Udenigwe & Aluko, 2012). The processing products of UF is a permeate and a retentate containing peptides smaller and larger than the filter`s pores size respectively. The filter`s pore size is usually specified by the molecular weight of the smallest peptides that are 90% retained from passing through the membrane, known as the molecular weight cut-off (MWCO) (Crittenden, Trussell, Hand, Howe, & Tchobanoglous, 2012). By selecting the appropriate MWCO, it is possible to concentrate peptides of various sizes depending on the desired properties of the product as illustrated in Figure 5. UF using 1-4 kDa MWCO membranes and low pressure (<500 kPa) has been applied to fish protein hydrolysates in several studies (Farvin et al., 2016; Girgih et al., 2015; Picot et al., 2010) including the laboratory experiments presented in this thesis. One of the major obstacles for using UF technology in the industry is to maintain an effective flow over the membrane by avoiding clogging (Bacchin, Aimar, & Field, 2006). Clogging is a common problem that results from the formation of a layer on the membrane consisting of components of the protein hydrolysate. Obtaining an efficient, stable, and reliable process is a requirement for industrialization of UF technology, which will

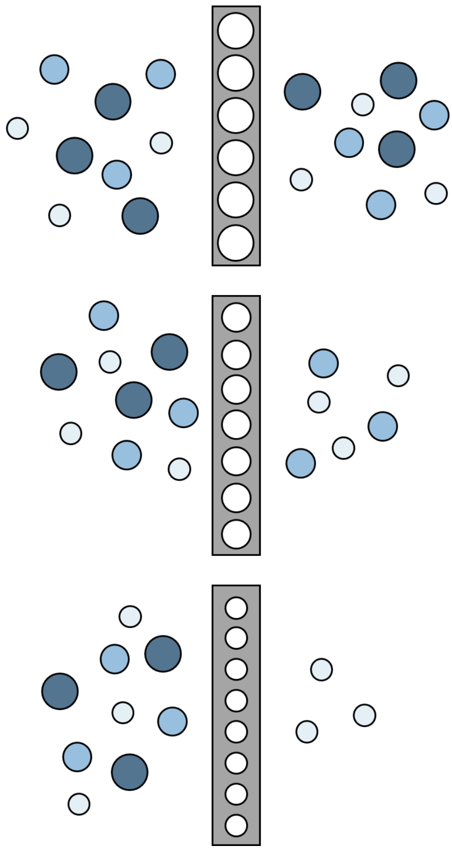

Figure 5: *Principles of membrane ultrafiltration using different molecular weight cut-off (MWCO). A large MWCO (top) allows more peptides to pass through the membrane from the retentate (left) to the permeate (right) compared to a small MWCO (bottom).*

necessitate an adaptation of the process to the raw material, the desired product and above all the industry.

7 A systemic and innovative approach

Research and development of biotechnological processing methods to improve utilization of RRM is undoubtedly important to make the whitefish industry more sustainable. Technological solutions are normally optimized based on the analysis of measurable outcomes from deductive and theory-driven research, which shows that quantitative analysis continues to be the dominating paradigm of natural sciences. However, these traditional lines of thinking might prove an inadequate premise when facing big environmental challenges. This thesis argue that a different approach is needed to understand the complexity of improving the utilization of whitefish RRM and develop the best solutions. As described in Paper I, an innovative approach that combines inputs from the laboratory, industry, it`s framework and stakeholders. Innovation is however a comprehensive term that has numerous more or less consistent definitions, which vary both between and within branches of knowledge (Baregheh, Rowley, & Sambrook, 2009). The lack of a consistent definition, as well as the complexity of those commonly used, can make it hard to understand what innovation is and what it describes. In that regard, the definition used by Halloran et al. (2020) in their Cookbook for systems change is an excellent example of how something complex can be described in a clear and simple form: "The term innovation means doing something differently and deliberately in order to achieve a certain objective". The objective of this thesis is to make the whitefish industry more sustainable by improving the utilization of whitefish RRM. Using an innovative approach therefore implies that I intentionally do something different, whether that is finding new methods or new combinations of already existing methods. It is also important to keep in mind that improved utilization of RRM is merely one part of the yellow brick road. Making the whitefish industry more sustainable involves transforming the way we utilize fish, from capture, through landing, processing, and distribution, and finally consumption. In other words, innovation is needed throughout the value chain.

A systemic approach involves taking a step back, seeing the bigger picture and identifying all the factors contributing to complex problems. This approach encourages a holistic perspective, which is a prerequisite to achieve a thorough understanding of the complexity and context of improving utilization of whitefish RRM (Halloran et al., 2020). Design thinking complements a systemic approach in that it challenges researchers to move the focus away from the technology to be developed, and rather towards the developmental process itself (Curedale, 2018). In these lines of thinking, innovation starts with the needs of the planet and its people. This involves reaching out, obtaining multiple perspectives and all relevant information including what might previously not have been considered important. One perspective that in my opinion is often wrongfully overlooked is the experienced-based knowledge of fishers. However, this knowledge can be combined with the quantitative knowledge of natural science in the open and inclusive process that a systemic approach facilitates (Jentoft, 2006). Instead of mere subjects of change, everyone involved in the whitefish industry should be thought of as a potential provider of a unique perspective of the challenges faced and how to best solve them (Halloran et al., 2020).

To what degree the experienced-based knowledge of fishers is currently incorporated into research and development, and drafting of laws and regulations, may vary and depend on several factors including social structures and preconceived attitudes. Fish scientist have been found to consider fishers as very knowledgeable regarding factual knowledge about fishing practice, but to a lesser degree of science in general (Bailey, Liu, & Davidsen, 2017). Having doubts abouts the fishers level of knowledge has also been identified among developers of fish processing equipment (Bar, 2015). Furthermore, fishers have been perceived as little receptive to inputs from scientist, and as dishonest about reporting accurate results of their activity (Bailey et al., 2017). Uncovering such underlying assumptions are highly relevant as the societal understanding of what type of knowledge fishers hold, and the value of this knowledge, is likely to affect the value it is attributed. Fishers' perspectives are more likely to be regarded as important if they are considered to be knowledgeable in the first place. Another relevant question is whether the fishers' role is merely a provider of information to complement scientific knowledge or as an active participant in research and development, management and drafting of regulations (Baelde, 2007; Jentoft, 2006). The knowledge obtained from a fisher as an active part of development is likely to be of a different nature than what is obtained merely as a provider of additional facts. As the information is no longer predetermined by identified gaps of existing knowledge, it can provide new insight that not only complement but fundamentally change the current understanding. Acknowledging the relevance and importance of fisher's experience-based knowledge, and how communication can be used as a data generating tool, is thus fundamental to understand the complexity of making the whitefish industry more sustainable by improving the utilization of whitefish RRM.

Obtaining a thorough understanding requires a search for knowledge about all internal and external factors that can affect the potential for improved utilization of whitefish RRM. Conventional quantitative methods are however not sufficient for analyses of the valuable information embedded in fishers` experienced-based knowledge obtained through formal and informal encounters (Baelde, 2007; Barclay et al., 2017). This insufficiency results from the nature of quantitative methods that make it impossible to acknowledge communication as a tool for obtaining empirical data, whereas written and spoken language is the primary empirical data in many qualitative methods (Braun & Clarke, 2013). By analysing written and spoken language, qualitative analyses seek to generate knowledge about how people understand and experience the world, and values both personal involvement and partial subjectivity. Qualitative analyses are primarily inductive research, meaning research that seeks to find patterns in empirical data recognized as gathered in a specific context, that can contribute to more general understandings or theories (Tjora, 2018c). An innovative and systemic approach might entail combining quantitative and qualitative methods in a new methodology toolbox. It might also involve developing new methods to fully understand the complexity and context of improving utilization of whitefish RRM. Maybe it is time to redefine what is relevant knowledge and what is the correct approach when attempting to create sustainable innovation.

8 Materials and methods

The first part of this thesis involves the use of biotechnological processing (Section 8.2) on saithe RRM (Section 8.1) for bulk production of protein products (Paper II) and the refinement of these to increase bioactivity (Paper III). Chemical analyses were conducted on the raw material and the processing products (Section 8.3). The obtained results were analysed statistically (Section 8.4). The second part of this thesis involves the use of qualitative analysis (Section 8.5) on eight interviews obtained from a case study within the Norwegian whitefish industry.

8.1 Raw material

A total of nine saithe (*Pollachius virens*) caught in Trondheimsfjorden, Norway, at two separate occasions were used for the experiment. Four pre-spawning saithe (2.1-3.4 kg, average weight: 2.7 kg) were caught in October 2019 and five spawning saithe (2.9-3.9 kg, average weight: 3.3 kg) were caught in January 2020. The fish were bled and kept on ice for transport, eviscerated and hand filleted in NTNUs Food processing laboratory. The raw material was separated into four main fractions: head (H), backbone (B), fillet (F) and viscera (V), which were vacuum packed and frozen at -40°C. The heads and backbones obtained in October (**H**ead **O**ctober, **B**ackbone **O**ctober) and January (**H**ead **J**anuary, **B**ackbone **J**anuary) were minced, then used for further processing and analysis.

8.2 Processing methods and experimental design

Two processing methods were used in this thesis: enzymatic hydrolysis and UF. Enzymatic hydrolysis was conducted on saithe RRM with the purpose of extracting its protein content. The processing conditions used for the enzymatic hydrolysis in this thesis are presented in Table 3 and further details are provided in Paper II.

Table 3: *Processing conditions of enzymatic hydrolysis of saithe rest raw material and membrane ultrafiltration of saithe protein hydrolysate.*

Processing method	Processing conditions
Enzymatic hydrolysis	
Instrument	Syrris Atlas Model No. 2101000 Bioreactor
Thermostat	Huber Ministat 125
Raw material:Water	1:1
Enzyme concentration	0.1% papain + bromelain
Duration	60 minutes
Temperature	50°C
Pressure	100 kPa
pH	Physiological
Membrane ultrafiltration	
Instrument	MMS Membrane Solution Triple System
Membrane I	Nadir® (150 kDa MWCO)
Membrane II	Nadir® UH004 P (4 kDa MWCO)
Raw material:Water	1:100
Average flux	50 L/m^2h
Temperature	21°C
Pressure	500 kPa
pH	Physiological

The enzymatic hydrolysis was conducted on raw material HO, BO, HJ and BJ in two bioreactors (I, II) as shown in Figure 6. Bioreactors were used as a step in the controlled upscaling from laboratory scale to pilot and industrial scale as described in Section 5 and Paper II. Three processing products were generated from enzymatic hydrolysis: an oil (lipid) fraction, a water-soluble fraction (protein hydrolysate) and an insoluble fraction (sludge). The saithe protein hydrolysate (SPH) was freeze-dried to a protein powder that was used for further processing.

UF was conducted on SPH for the purpose of separating peptides based on size and concentrating small peptides to increase the antioxidative activity of the obtained product. An initial UF using a membrane with 150 kDa MWCO was conducted to remove impurities. This generated two processing products: a retentate with peptides >150 kDa (R150) and a permeate with peptides <150 kDa (P150). An UF using membranes with 4 kDa MWCO was further conducted on P150, generating a retentate with peptides >4 kDa (R4) and a permeate with peptides <4 kDa (P4). The processing products generated from both filtrations are shown in Figure 6. The processing conditions used for UF are presented in Table 3 and further details are provided in Paper III.

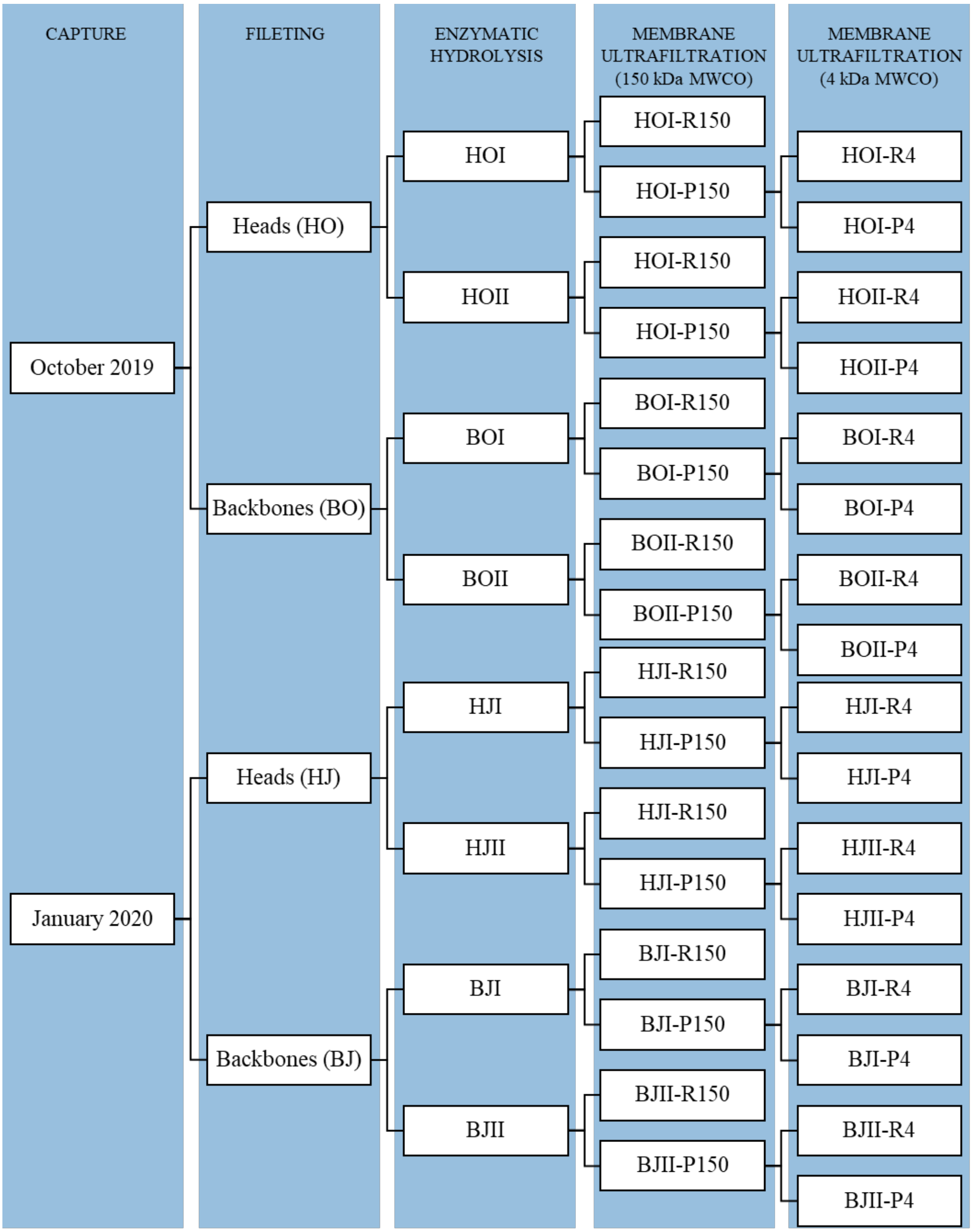

***Figure 6**: Experimental design and overview of the processing products generated from capture, fileting, enzymatic hydrolysis of saithe rest raw material in two (I, II) bioreactors, and two sequential membrane ultrafiltrations of saithe protein hydrolysate.*

8.3 Chemical analyses

A summary of the chemical analytical methods used in this thesis is presented in Table 4. Further details can be found in the indicated papers.

Table 4*: Overview of the chemical analysis conducted in this thesis with indication to Papers and associated reference(s).*

Chemical analysis	Description	Reference
Dry matter and ash content	Paper II, Paper III	AOAC (1990)
Crude protein	Paper II	Kjeldahl (1883)
Total lipid content	Paper II	Bligh and Dyer (1959)
Degree of hydrolysis	Paper II	Taylor (1957) and Kvangarsnes et al. (2021)
Molecular weight distribution	Paper II, Paper III	Innolipid AS
Colour measurement	Paper II	
ABTS radical scavenging activity	Paper III	Re et al. (1999) and Nenadis, Wang, Tsimidou, and Zhang (2004)
FRAP assay	Paper III	Benzie and Strain (1996)
ORAC assay	Paper III	Dávalos, Gómez-Cordovés, and Bartolomé (2004) and Jensen et al. (2009)
Amino acid composition	Paper III	Blackburn (1968)

8.4 Statistics

All statistical analyses were conducted in SPSS software (IBM SPSS Statistics 27). Analysis of variance (ANOVA) and T-test were used for comparison of means, assuming normal distribution and equal variance. Tukey`s post hoc test was used in combination with ANOVA to identify significant differences. Significance level was set to $p < 0.05$. Results are reported as mean values ($\overline{x}$) of [n] number of parallels ± standard error of the mean (SEM).

8.5 Qualitative analysis

A case study was conducted in the Norwegian whitefish industry with the goal of investigating how experiences, attitudes and practices among fishers, and the factors affecting them, could enable or complicate efforts to improve utilization of RRM. Qualitative methods were used for both the collection and analysis of the data material.

8.5.1 Data material

Collection, storage, and processing of the data material was performed according to the ethical guidelines of the Norwegian Centre for Research Data (NSD). The data material consisted of eight in-dept interviews with fishers of various work positions on vessels of the Norwegian coastal fleet. In-depth interviews were conducted with the aim of facilitating an open conversation where the participants reflected on their own thoughts and meanings about open questions (Tjora, 2018a). The questions were based on an interview guide that had been prepared in advance (Appendix I). This type of data generation is particularly useful to study opinions and understandings among the participants and was thus determined the best option with regards to the aim of this study. The recruitment process for the interviews was long and based on volunteer involvement from participants after invitation through meetings, phone calls or email correspondence. Before the interviews, all participants received oral and written information about the study and provided their informed consensus. The interviews were conducted in the winter months of 2020/2021 during the peak harvesting season of whitefish. Six were conducted onboard a fishing vessel while the remaining two were conducted over video call. The duration of the interviews was between 30 and 60 minutes.

8.5.2 Thematic analysis

Thematic analysis was used to identify, analyse, and present patterns within the data collected from the eight interviews. This analytical method does not require detailed theoretical knowledge, nor is it limited by a pre-existing theoretical framework, which makes it an accessible form of qualitative analysis (Braun & Clarke, 2006). Thematic analysis also acknowledges the active position of the researcher in identifying patterns across data as opposed to themes emerging from the data themself. The coding process was data-driven and inductive, aiming for a bottom-up analysis. The data material was approached with an emphasis on how each individual makes sense of their experiences and how this is influences by the social context they are in (Braun & Clarke, 2013). Themes were identified at a latent level, meaning that underlying ideas, assumptions, and conceptualizations beyond what was explicitly stated in the data material was identified.

8.5.3 Data analysis

All interviews were recorded using an audio recorder (Sony ICD-PX370). The audio files were transcribed in a verbatim fashion. Dialect and slang words were replaced with Norwegian written language, while personal and geographical names were replaced with general descriptive terms. This was done in order to anonymize the data material as well as standardizing it for the subsequent analysis. The data material was further anonymized by removing any personal information and renaming files participant 1-8 (P1-P8). The data material was coded using NVivo 1.3(535) Software. The initial coding generated 160 codes, which were categorized and re-categorized repeatedly until it was possible to create and define three main themes.

The quotes given in this thesis are translated from Norwegian. The translation process was conducted with a goal of maintaining the words and sentence structure as close to the original as possible, and thus might appear grammatically challenging to read in English. Quotes are initiated and terminated by quotation marks ("), while […] indicates that something has been removed.

8.5.4 Limitations of the method

The quality of a qualitative analysis depends on its reliability, validity, and generalizability (Tjora, 2018b). It is by no means useful to argue that my prior knowledge or personal attitudes did not affect the findings of this study. In fact, the active role of the researcher, and some level of researcher subjectivity, is both an inevitable and acknowledged part of a qualitative analysis (Braun & Clarke, 2006; Tjora, 2018c). The same is true for the participants, for whose answers to specific questions might depend on both understanding, the interview context, and the fact that RRM is a controversial topic. However, both the interview process and the data analysis were conducted with the ambition of remaining as objective and open-minded as possible. The themes identified has provided insight that is highly relevant for the aim of this thesis. However, as this analysis was a case study from a contextualism viewpoint, it follows that the knowledge obtained from these results are predominantly valid within the context they were produced – a narrow part of the Norwegian whitefish industry. Some level of generalizability is still likely, and these research findings should be considered as a part of future research within both local and national fisheries.

9 Results and discussion

The results presented in this thesis consist of three main parts:

Part I (Section 9.1) presents results from laboratory experiments conducted to evaluate the effect of spawning on saithe RRM, the potential for upscaling biotechnological processing for bulk production of protein products and the refinement of these to increase bioactive properties.

Part II (Section 9.2) presents results from the case study and the analysis of eight interviews with fishers of the coastal fleet.

Part III (Section 9.3) present the factors that could affect the potential for improved utilization of whitefish RRM that were identified based on a combination of the insight obtained from the laboratory, observations, and interviews.

9.1 Biotechnological processing of whitefish rest raw material

RRM consisting of heads (H) and backbones (B) from saithe caught in October (pre-spawning, O) and January (spawning, J) were minced and processed by enzymatic hydrolysis in two bioreactors (I, II) creating a total of eight (HOI, HOII, BOI, BOII, HJI, HJII, BJI, BJII) SPH (Section 8.2, Figure 6). Of the RRM fractions head, backbone, and viscera, only viscera were found to vary significantly in size between pre-spawning and spawning saithe (Paper II). Spawning neither affected the nutritional composition of heads and backbones, nor the quantity, quality, or bioactivity of SPH. Varying size and composition of RRM can be problematic for industrial processing that require a uniform production and a continuous product supply to the market. Viscera is also a highly perishable RRM compared to heads and backbones due to its higher content of microorganisms, endogenous enzymes and lipids prone to oxidation (Rustad et al., 2011). If mixed with heads and backbones, viscera could reduce the stability of these RRM fractions as well. This thesis thus argues that sorting of RRM will be a requirement to maintain quality and enable bulk productions of protein products for human consumption. Viscera can however be used for extraction of highly refined products such as omega-3 fatty acids and enzymes, or for production of animal feed and biofuel (Daukšas, Falch, Šližytė, & Rustad, 2005).

The focus of this thesis was not to evaluate processing parameters of the enzymatic hydrolysis, but rather the functionality of processing in bioreactors as the first step in a controlled upscaling from lab scale to pilot and industrial scale (Section 5, Figure 4). Mincing of RRM prior to processing is beneficial for obtaining a homogenous reaction mixture and facilitates the hydrolytic activity of enzymes by exposing more of the protein content (Šližytė et al., 2009).

Heads and backbones do however have a high content of bone structures, which made processing challenging and was found to require a powerful mincer with a high cooling capacity compared to industrial meat mincers (OMAS Meat Mincer Tritacarne TS 22E). A need for powerful processing equipment adapted to the RRM was also identified to achieve sufficient stirring in the bioreactors, as discussed in Paper II. Enzymatic hydrolysis separated the RRM protein content into SPH and an insoluble sludge fraction. The protein yield in SPH was found to be significantly higher for backbones (39.9 ± 2.5% (BO), 39.3 ± 1.3% (BJ)) compared to heads (28.4 ± 3.4% (HO), 34.7 ± 0.3% (HJ)).

SPH in its initial form was a liquid solution with 5 - 8% dry matter, consisting mainly of protein. In an industrial setting, it is however essential that the water content is removed to increase stability and reduce weight and volume of SPH. Freeze-drying, as used for dewatering in this thesis, is a time-consuming and expensive process that might not be economically viable for industrial purposes (Petrova, Tolstorebrov, & Eikevik, 2018). This thesis thus identifies it as necessary to find fast and energy-efficient options for industrial dewatering of SPH. Freeze-dried SPH had the texture and appearance of a white protein powder as shown in Figure 7.

Figure 7: *Freeze-dried saithe protein hydrolysates (SPH) obtained from enzymatic hydrolysis of saithe heads (H) and backbones (H) in two bioreactors (I, II) from saithe caught in October 2019 (HOI, HOII, BOI, BOII) and January 2020 (HJI, HJII, BJI, BJII).*

Good quality SPH for human consumption should have a high content of digestible protein, balanced amino acid composition, low lipid content and high whiteness (Dale, Madsen, & Lied, 2019; Kristinsson & Rasco, 2000; Šližytė, Rustad, & Storrø, 2005). The quality parameters of SPH are presented in Table 5. All SPH were found to be of good quality as described in Paper

II, with SPH from backbones having a significantly higher protein content and whiteness compared to SPH from heads. The quantity and quality of SPH was also found to be comparable to those obtained from similar processing of cod RRM. No ideal value for the degree of hydrolysis (DH) is included in Table 5, as the desired DH of SPH depends on the intended areas of application. A low DH indicates a higher fraction of larger peptides in SPH, while a high DH indicates a higher fraction of smaller peptides in SPH. Larger peptides have better functional characteristics, like emulsifying, foaming and water binding properties, and sensory characteristics, like taste, which is beneficial for SPH as a food ingredient (Daukšas, Šližytė, Rustad, & Storro, 2004; Gbogouri, Linder, Fanni, & Parmentier, 2004; Halim, Yusof, & Sarbon, 2016; Karami & Akbari-Adergani, 2019). Bitterness is an undesired property of SPH that can be reduced by limiting DH and adding antioxidants (Halldorsdottir, Sveinsdottir, Gudmundsdottir, Thorkelsson, & Kristinsson, 2014). However, as discussed in Paper III, bioactivity is associated with peptides of 3 - 20 amino acids and smaller peptides might thus be desirable if the intended use for SPH is as a source of bioactive peptides (Zamora-Sillero et al., 2018).

Table 5: *Protein content (% SPH dry weight, $\bar{x}$ ± SEM, n = 3), PER-value, lipid content (% SPH dry weight, $\bar{x}$ ± SEM, n = 2), DH (%, $\bar{x}$ ± SEM, n = 3) and whiteness ($\bar{x}$ ± SEM, n = 3) of saithe protein hydrolysates (SPH) obtained from enzymatic hydrolysis of saithe heads (H) and backbones (H) in two bioreactors (I, II) from saithe caught in October 2019 (HOI, HOII, BOI, BOII) and January 2020 (HJI, HJII, BJI, BJII).*
**Calculated as described in Paper III, with PER of cod muscle chosen as an ideal value (Šližytė, Daukšas, Falch, Storrø, & Rustad, 2005).*
***Calculated as described in Paper II.*

Sample	Protein (%)	PER*	Lipid (%)	Whiteness**	DH (%)
Ideal value	100.0	3.0	0.0	100.0	
HOI	90.3 ± 0.1	1.6	3.6 ± 0.2	74.8 ± 0.4	17.2 ± 0.2
HOII	91.4 ± 0.1	2.0	3.1 ± 0.4	76.8 ± 0.1	18.1 ± 0.1
BOI	94.3 ± 0.5	1.3	3.5 ± 1.6	81.9 ± 0.1	17.9 ± 0.1
BOII	94.1 ± 0.1	1.3	1.7 ± 0.1	81.7 ± 0.1	17.5 ± 0.1
HJI	91.1 ± 0.1	1.9	1.7 ± 0.8	79.1 ± 0.1	14.8 ± 0.1
HJII	87.4 ± 0.1	2.0	1.9 ± 0.5	77.7 ± 0.3	16.3 ± 0.0
BJI	96.1 ± 0.1	1.4	0.9 ± 0.9	79.1 ± 0.1	17.5 ± 0.1
BJII	96.3 ± 0.1	1.5	0.0 ± 0.0	81.5 ± 0.1	18.0 ± 0.3

UF was conducted on SPH in order to concentrate small peptides to potentially increase the bioactivity of the processing product. An initial UF using a 150 kDa MWCO membrane was conducted on SPH to remove impurities and obtain a better separation in the subsequent UF using 4 kDa MWCO membranes. UF on SPH with a 150 kDa MWCO membrane separated the peptides in a retentate (R150, >150 kDa) and a permeate (P150, <150 kDa), while the subsequent UF of P150 created a new retentate (R4, >4 kDa) and permeate (P4, <4kDa). An overview of the different processing products generated from UF processing can be found in Section 8.2, Figure 6. The antioxidative activity of SPH, retentates, and permeate were analysed using a combination of three assays, as described in Paper III. Under the assumption that a higher concentration of small peptides would yield a higher antioxidative activity, the expected results for UF processing products would be P4>R4>R150. SPH would also be expected to exhibit lower antioxidative activity due to the relatively lower concentration of small peptides compared to P4. UF effectively concentrated small peptides in P4 as shown in Figure 8.

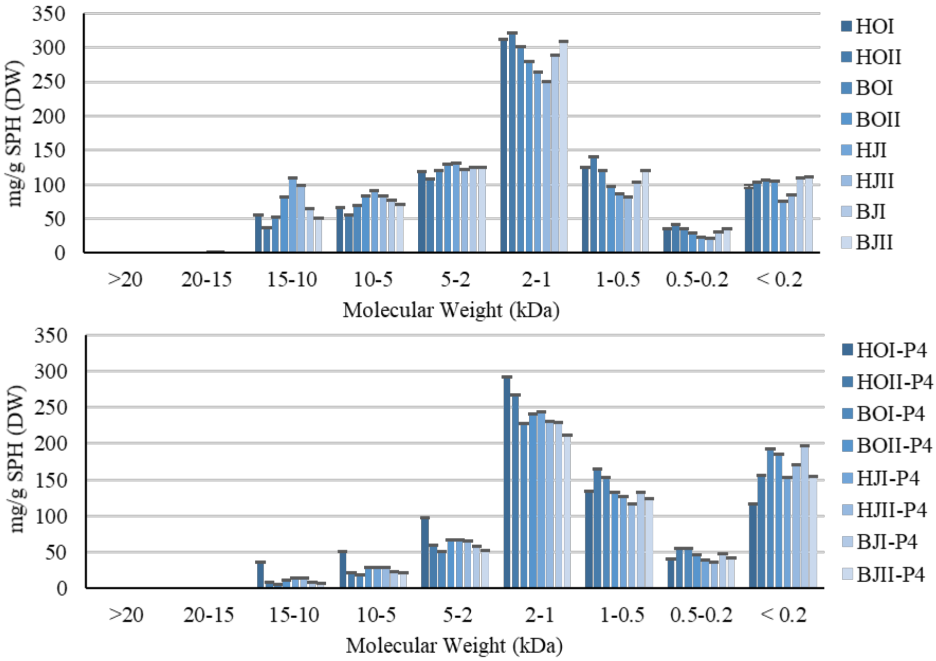

Figure 8: *Molecular weight distribution ($\overline{x} \pm$ SEM, n = 2) of saithe protein hydrolysates (SPH) (top graph) and their corresponding permeates (bottom graph) after membrane ultrafiltration (P4, <4 kDa). SPH were obtained from enzymatic hydrolysis of heads (H) and backbones (B), in two bioreactors (I, II), from saithe caught in October 2019 (O) and January 2020 (J).*

The unfiltered SPH was however found to have a similar or higher antioxidative activity compared to P4 (Paper III). When evaluating the potential of UF processing, it is important to consider that any additional step added to an industrial processing line means added cost to the production (Kristinsson & Rasco, 2000). In addition to being a time-consuming and sensitive processing method, industrial applications of UF would require an investment in expensive equipment and expertise. This means that UF should give the obtained product a substantial value-addition to make this processing economically viable. Bioactive peptides could target a high-value market as pharmaceuticals or nutraceuticals, but based on the results presented in Paper III, more studies must be conducted on UF to justify the use of this technology in an industrial setting. However, the unfiltered SPH could be used as an ingredient in food to extend its shelf-life by preventing oxidation (Gao et al., 2021). Such application would reduce processing cost by eliminating the need for UF, while simultaneously avoiding the comprehensive process needed for approval of a health claim (Section 4, Figure 2). Based on the obtained knowledge presented in this thesis and available literature, it is my opinion that the most industrially relevant application of saithe RRM is bulk production of products that could be used as protein supplement or as an ingredient in food to improve functional properties and/or extend shelf-life. Processing remains rich in bone structures could subsequently be used for production of gelatine. Enzymatic hydrolysis and UF separated the protein content of saithe RRM in several different processing products as presented in Figure 9.

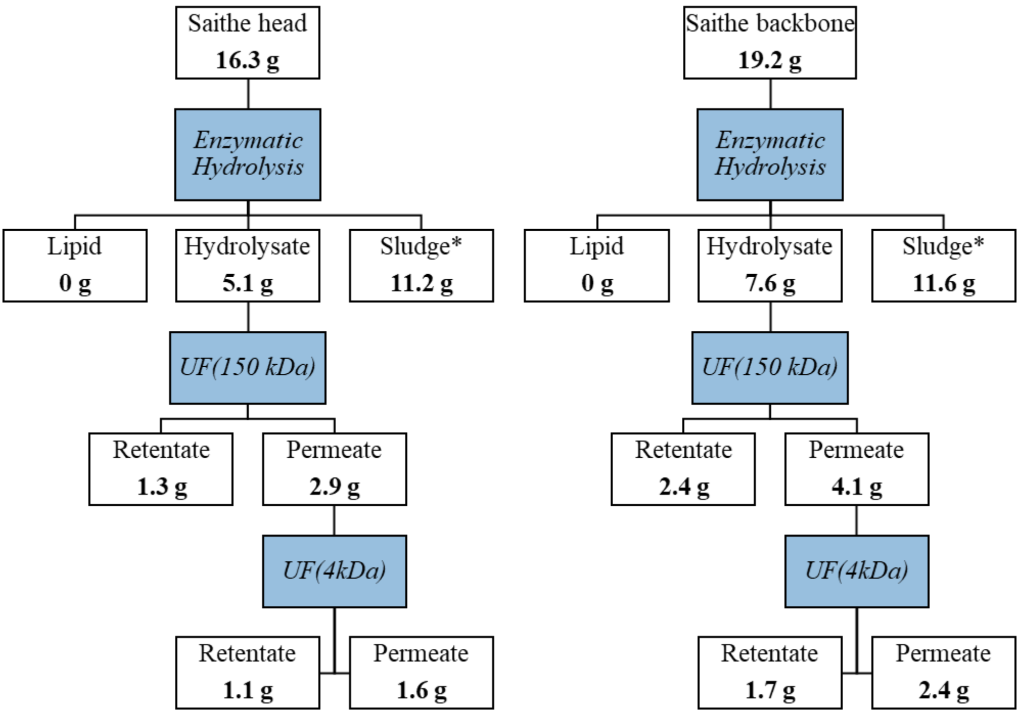

Figure 9: *Protein flow (g) from rest raw material to product after processing of 100 g minced saithe heads (left) and backbones (right) by enzymatic hydrolysis and membrane ultrafiltration (UF) using a 150 kDa MWCO membrane followed by 4 kDa MWCO membranes.*
**Calculated value.*

An improved utilization of whitefish RRM must be sustainable both for the economy and the environment, which means that it is also important to consider the total waste reduction after processing. The distribution presented in Figure 9 shows that a large proportion of the protein ends up in the secondary products of processing: the sludge from enzymatic hydrolysis and the retentates from UF. As presented in Paper II, more than 50% of the proteins, lipids, and minerals from RRM end up in the sludge fraction. Considering that as little as 30% and 10% of the RRM protein is recovered in the SPH and P4 respectively, it would be highly wasteful to solely use these processing products. It is thus necessary to evaluate the potential application of secondary processing products as well. The sludge fraction could be used for animal feed, but also extraction of gelatine for human consumption (Araujo, Sica, Costa, & Márquez, 2020; Gildberg et al., 2002). The extracted gelatine could be used as a gelling ingredient in food, but has also been found to exhibit bioactive properties (Lv et al., 2019). The larger peptides of the retentates could be utilized for their functional properties as emulsifying, foaming or water binding ingredients in food, as described above. Hofseth Biocare (HBC) is an example of a Norwegian actor that markets a range of commercial products from salmon RRM, including the protein hydrolysate product ProGo®, calcium bone powder CalGo® and collagen peptide CollaGo® (HBC, 2021).

9.2 A case study of the Norwegian whitefish industry

Analysis of the eight interviews with fishers of various work positions on vessels of the Norwegian coastal fleet, resulted in the creation of three main themes. The first theme concerns the term sustainability and how its interpretation, which also involves the fishers` perception of RRM, can affect rationalization, behaviour, and attitudes. The second theme concerns the value chain, its fragmented organization and how this affects the fishers. The third theme deals with regulatory authorities and regulations, how they are developed and implemented, and their implications for the fishers.

9.2.1 Theme 1: “All is well with the fish” - The incomprehensible term sustainability

When asked about the term sustainable utilization of marine resources, most of the participant directly connected sustainability with a good management of the fish stocks: “Yes, you are thinking about environment-related sustainability? Then we are talking about resource outtake (P1)”. Further reasoning about stock management seemed to be centred around a common understanding that it is in the interest of everyone to maintain a good management, that “Stock monitoring is good (P7)” and that the quota system aids to regulate fishing activities to avoid overfishing: “But it is important that there is, to have quota on things, that a decent fish stock is maintained (P5)”. The term sustainability was also found to be interpreted differently, and given different meaning, among developers of fish processing equipment (Bar, 2015). This indicates that what is understood by sustainability is a product of the context in which it is used, altering the term diffuse and, to varying degrees, incomprehensible. From a systemic point of view, good stock monitoring is merely a part of sustainable utilization of marine resources. Other aspects important to consider include how the fish is processed and transported, how much RRM is generated and to what degree it is utilized. The sustainability benefits of improving the utilization of RRM can in many ways be thought of as a key element to justify regulation like the landing duty and financing research and development of RRM processing. The question then becomes how clearly these other aspects are communicated to those affected by the push towards a more sustainable industry. When used to explain or justify regulations, actions, or desired changes of practices within the whitefish industry, incomprehensive terminology might cause confusion and frustration. As mentioned above, the participants identified their role in sustainable utilization as ensuring a good resource outtake, but beyond that, most participants struggled to see their role in further improving the utilization of fish. Without a clear understanding of why it is beneficial and important to take care of RRM and make the most out of the fish, and what role these first steps of the value chain have in this action, it is understandable that regulations and demands can be perceived as stressful and useless. A lack of perceived importance in relation to the overall environmental impacts, was also identified as a potential obstacle for sustainable development within the fish industry by Bar (2015).

Regardless of the term sustainability, there seemed to be a consensus among the participants that the fish is our shared resource of the ocean, one that we all need to take care of in order to secure food and workplaces for the future: “We must not use so much that we ruin for next year, and we have to deliver what we catch (P4)”. This is in line with theories suggesting distribution of authority as a naturally emerging option for government of common-pool resources, in contrast to a need for privatizing or having it controlled by a central authority (Ostrom, 2010). Applied to the whitefish industry, such theories suggest that in the event of having to share a common-pool resource like fish, fishers will form locally initiated regulations. Enforcement of these regulations will be based on mutual trust and a common interest to safeguard the resource. Most participants were conscious of, and made reflections about, their responsibility in this regard. This highlights how motivation to comply with regulations is affected by the individual's sense of responsibility, the basis for regulations and the perceived feeling of being a part of their implementation. As mentioned, the quota system was addressed by the participants as something that can be relied upon, a system set in place to secure and safeguard the balance of the ocean. It might be that the positive association around quota regulation comes from the fact that it is perceived as being part of an inclusive bottom-up regulation as opposed to an exclusive top-down regulations. A regulation that the fishers have had a part in developing, sees the advantages of and their responsibility in ensuring compliance.

Another important aspect identified as part of the first theme was how RRM was perceived by the participants, and whether it was considered valuable or not. Regulations, research, and development focusing on new ways to utilize RRM for production of high-quality ingredients for food and feed inevitably entails taking RRM away from traditional ways of usage. This imposed change of practice was problematized by several of the participants. The fishers live in symbiosis with the fauna and flora of the ocean, a mutual understanding of function and demand that stretches centuries back in time. When a fish is taken from the water, the viscera go back into the ocean to feed "the sea bird […] which live mostly of what we used to throw into the sea (P7)" and for "fertilization of the seabed (P6)". The RRM already have a function in the traditional practice, it is given back to the ocean as part of a holistic utilization. Although it has been pointed out that fishers opposition is often perceived as resistance to change and lack of care for the environment by environmentalist (Baelde, 2007), a lack of care was not identified in this case study. None of the participants sympathized with unnecessary dumping of marine resources. Rather, it is important to consider that giving a new and modern value and function to a product that already has an existing role might disrupt traditional core values and practices, and thus work as an obstacle for change. The change of fishing practice associated with improving utilization of RRM also seemed to be highly connected to economical and practical considerations, ultimately leading to a "[…] cost-value evaluation (P1)" among the participants. Although many of the participants were aware of the potential value that lies within improved utilization of RRM, it was looked at with scepticism as to the time-efficiency and financial sacrifices having to be made: "It is going to take a lot more time […] no, that would be cumbersome […] then one would have to have a space to keep the liver and the roe […] (P5)". In addition, it seems to be hard to grasp what actual value the viscera can have besides being sought after for its roe and liver niche products during the peak spawning season in January – April: "During the rest of the year there is no roe in the fish, and the liver, it has no use then (P2)" and for the fishers: "We have no use for them (P3)". Besides getting paid for roe and liver during the peak harvesting season, fishers do not get any economical compensation for bringing the RRM to shore, as they are obliged to according to the landing duty. This points to a strong connection between motivation to conserve RRM and economy, and that economic incentives could be used as a tool to improve utilization of RRM. It is thus evident that the perceived importance of RRM, the fisher's role in sustainable utilization of RRM, the rationale behind utilizing it in new ways and the incentives ensuring such practices, can affect the potential for improved utilization.

9.2.2 Theme 2: "It is us and then there is them" - The fragmented value chain

The second theme generated in this study was related to the functionality of the whitefish value chain and how one step relates to the next. When asked about the value chain, or how further processing of the fish was organized, the answers received from the participants reflected on a series of events that cannot be considered a dynamic and continuous, but rather a fragmented and divided process: "You are thinking of our part, or what happens after that (P5)?" The lack of a continuous value chain, where everyone feels responsible for the products from beginning to end, results in the illusion of a separation between the different steps and stakeholders. This manifests itself in an understanding of that it is "us" and then there is "them": "My work stops when I have delivered it. Put it on the quay and they pick it up […] and they sell it on to buyers who sell it on to consumers (P1)". A fragmented value chain can thus lead to a rightful disclaimer by the fishers as to what happens to the fish and the RRM after it is delivered to the landing facilities. Several participants also expressed that even though their practice ensured high quality of the fish, these efforts seemed pointless when the quality was not maintained or considered important throughout the value chain. The "us" and "them" can quickly evolve to "us" against "them", and create feelings of injustice, when other stakeholders of the value chain are suspected to make money on behalf of earlier steps. "They" walk away with the profits of utilizing RRM: "So depending on what they do with it, that I don't know, depending on if they make filet or what products they make out of it, but. So, it is they who have run off with the largest pot usually (P2)" when all the pressure of bringing it to shore is put on "us": "[…] harvesting stage and landing facilities […] I feel that in many cases it is these two steps that are being put off with crumbs (*Norwegian expression meaning trying to satisfy a counterparty with something that does not meet their requirements or expectations*) (P6)". The fragmented value chain, and the unnatural divisions it creates and amplifies between the different stakeholders of the whitefish industry, can thus be a hurdle for improving utilization of RRM. It can also conceivably affect the general quality of the fish in a negative way, which is decisive for the quality of both filets and RRM processing products. If each individual considers themselves released from responsibility when their task is completed, and the fish is delivered to the next step of the value chain, it can be hard to maintain control of both the generation and quality of RRM. The RRM is assumably handled by "them", so "we" need not to worry: "[…] that is not our table anymore (*Norwegian expression meaning not our responsibility*). We have, our job is just to get the fish ashore (P7)". As such, the fragmented value chain can have a considerable influence on the perceptions, attitudes, and opinions amongst stakeholders of the whitefish industry and thus affects the potential for improved utilization of RRM.

9.2.3 Theme 3: "We are not seen or heard" - The loss of recognition

The third theme generated was the fishers perceived loss of recognition by regulatory authorities and consequently a feeling of not being seen or heard. The participants reflections pointed to a perceived loss of recognition both as an important contributor to food production, and as a holder of experienced-based knowledge about fishing practices and "how things are best done". Experience-based knowledge that has been passed on through generations: "That I have learned from my father. And father has learned it from the old folks that were carrying on at that time (P2)", and that should rightfully be taken into account in order to develop systems and regulations that function beyond their theoretical ambitions. Such an understanding harmonize with the view of experienced-based knowledge as a cornerstone in the pride fishers take in their occupation (Baelde, 2007; Jentoft, McCay, & Wilson, 1998). A pride of something they *are* rather than something they *do* for a living.

Another aspect to consider regarding this theme is to what degree the regulatory authorities are perceived as taking the experience-based knowledge of fishers into account when developing new laws and regulations. If the reasoning and logic behind regulations is difficult to comprehend, and they are not perceived as giving any sense or meaning to those affected by them, it can amplify the feeling of not being seen or heard: "[…] they who decide a lot of the rules, they have no idea what they are talking about […] it is not feasible (P2)". When new regulations come into conflict with practical work, economic considerations, or moral values of the fishers, they might thus act as a hurdle for improving utilization of RRMs: "For us to bring it ashore, that is no problem, but I just don't see the point (P6)". A quickly changing regulatory framework, that might involve only minor adjustments in the written statements, can also have huge impact on the daily practice for fishermen. Such changes can further contribute to a scepticism towards regulatory authorities: "And then we had to buy a new set of quotas to fill up, but then the price was almost ten times as high as it was six to eight years back. We had to pay ten times what we sold for. So therefore, I am very sceptical to politicians and all sorts of regulations (P7)". In contrast to the participants positive view of the quota system as a tool for appropriate stock monitoring (Section 9.2.1), the quickly changing regulatory details seemed to give a perception of a more fluctuating and unpredictable system among the fishers. A system controlled by regulatory authorities, who would alter it at their own benefit without considering the effects such alterations could have for the fishers. One approach to increase the recognition of those affected by the system could be to increase the influence of fishers knowledge in developing the quota recommendations, which could benefit both the aquatic fauna and the sustainability of the fishing industry (Baelde, 2007).

When asked about the regulatory framework in general, several participants shared experiences with law execution and measures taken by local authorities to ensure compliance with existing regulatory framework. Amongst others, these experiences included distressing encounters with the coast guard and other authorities responsible for ensuring compliance with the landing duty. One participant described a feeling of being made a scapegoat due to the nature of these encounters. Some level of monitoring is usually necessary, and an effective mean, to enforce law. However, there seemed to be a consensus among the participants that current monitoring practices made regulations feel like a straitjacket that forced them into new practices that they neither have the necessary resources for, are prepared for, nor have had time to adapt to. As states by one of the participants: "It's not uncomplicated to say you simply cannot do it like this anymore (P6)".

If regulatory authorities are not perceived as taking into account the fishers' opinions, requirements and experienced-based knowledge this might negatively affect the motivation to accept and comply with new regulations. On the other hand, motivations would likely be positively affected if the development of regulations that aims to increase sustainability is performed as a systemic process that invites and includes all affected parties. A feeling of being included in a common goal to make the whitefish industry more sustainable and an understanding of the regulations as a necessary measure in that regard, as discussed in Section 9.2.1. Equally important is the potential benefits regulatory authorities could have from a systemic approach that to a larger degree include fishers, resulting in the development of a regulatory framework that is highly functioning both theoretically and practically. Such an approach would require good communication rooted in a mutual respect for each other's knowledge, which is likely to be improved by facilitating encounters across the value chain

(Bailey et al., 2017). Regulatory authorities, developmental processes for regulations and how they are implemented can thus affect the potential for improved utilization of RRM.

9.3 The potential for improved utilization of whitefish rest raw material

RRM contains the same nutritional components as the filet we eat for dinner, including high-quality protein. This protein can be extracted into products suitable for human consumption using the processing methods enzymatic hydrolysis and UF as shown in Figure 10. The quantities of whitefish RRM produced annually in Norway contains enough protein to cover the yearly requirements of 2.5 million adults. Today however, RRM protein is used for animal feed, biofuel, or simply wasted. Numerous studies over the last decades have identified optimal harvesting, storage, and processing conditions in order to produce high-quality food products from fish RRM. Even though the utilization of whitefish RRM has improved over the last years, the industrial practice and technology still reflects on a divergence in the level of development amidst research and industry. The question then remains, what is needed to reduce waste and produce a higher proportion of products for human consumption to improve the utilization of RRM.

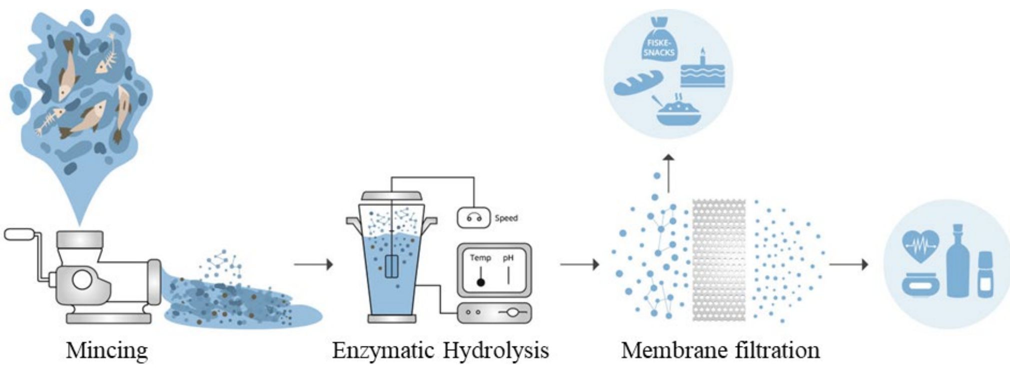

Figure 10: *Biotechnological processing of saithe rest raw material by enzymatic hydrolysis and membrane ultrafiltration generate protein products suitable for human consumption.*

This thesis argue that an innovative and systemic approach is needed to fully understand the complexity of improving the utilization of whitefish RRM and developing the best solutions to achieve this objective. An approach that combines inputs from laboratory experiments (Section 9.1), the whitefish industry and the experience-based knowledge of fishers (Section 9.2). A methodology combining quantitative and qualitative methods were used to identify internal and external factors that could affect the potential for improved utilization of whitefish RRM. The major findings are summarized in Figure 11.

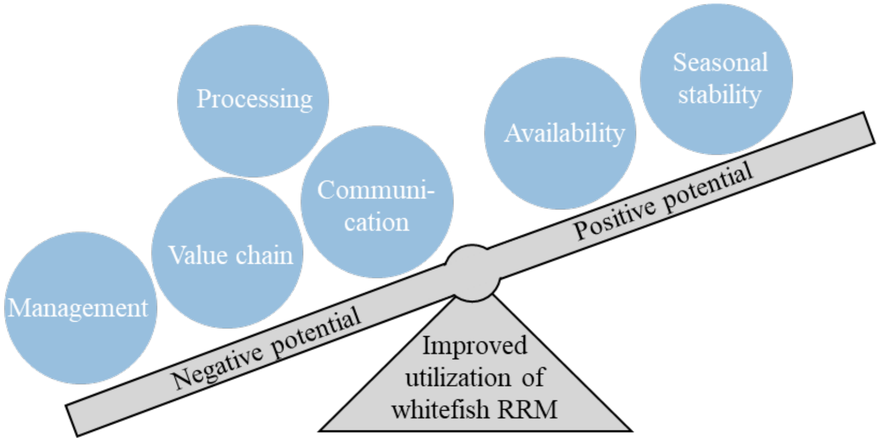

Figure 11: *Factors that could affect the potential for improved utilization of whitefish rest raw material (RRM). The individual factors tip the scale to the left (negative potential) or right (positive potential) depending on leverage.*

The individual factors identified in this thesis tip the scale in Figure 11 towards an overall negative potential, no potential, or a positive potential depending on leverage. The lack of appropriate and industrially relevant technology, like powerful and energy-efficient equipment for mincing, agitation, and dewatering, can make processing a factor with a negative impact on the potential for improved utilization of whitefish RRM (Section 9.1). In working towards a common goal, it is fundamental that all those involved have the same understanding of what that goal is. The use of complicated language and terminology, as discussed in Section 9.2.1, can thus make communication a second factor with a negative impact. Another issue regarding the logistics of the whitefish industry is the fragmented value chain, as discussed in Section 9.2.2. A fragmented value chain can lead to a disclaimer by the fishers as to what happens to the fish and the RRM after it is delivered to the landing facilities, which in turn might affect traceability. As mentioned in Paper I, increasing traceability could improve the ability to maintain a quality of RRM that satisfies the requirements for food production. Increased traceability could also lead to a better control over where in the value chain RRM is generated. The current organization of the value chain can thus present a third factor with a negative impact. As discussed in Section 9.2.3, the management strategies of the whitefish industry can create tension between the different stakeholders and consequently present a fourth factor with negative impact.

Spawning neither affected the composition of saithe RRM, nor the quantity, quality or antioxidative activity of the obtained processing products (Section 9.1). This could be highly beneficial for the use of heads and backbones in bulk production of protein products for human consumption, and seasonal stability could thus present a factor with a positive impact on the potential for improved utilization of whitefish RRM. The second factor that could have a positive impact is the availability of the RRM. The supply of RRM could also be further increased by moving more of the processing to Norway, as discussed in Paper II. Together with an improved utilization of RRM, this would contribute to make the whitefish industry more sustainable both from an environmental and an economic perspective.

To tip the scale in Figure 11 towards an overall positive potential for improved utilization of whitefish RRM, at least one of two things need to happen. Either more factors with a positive impact must be added or some of the negative factors must be made positive. The latter could be possible for e.g. management by adopting a more inclusive process for development of laws and regulation that take into consideration the opinions and experience-based knowledge of fishers. There are also several other factors that must be considered, as market and consumer acceptance of food products produced by RRM (Paper I). After numerous formal and informal conversations with various stakeholders of the whitefish industry, it is however my opinion that the main problem for improving utilization of whitefish RRM is the lack of incentives. A lack of economic and other incentives for fishers to bring the RRM to shore, but also for the industry to change production from feed to food. The current management consist of regulations that forces through a change of fisher practice without any incentives to do so. Large proportions of the RRM are however generated further down the processing lines, which is also where the value-addition of RRM takes place. The strain is put on the fishers, but the profit goes to the producers. An economically viable production is undeniably a premise for the processing parts of the industry as well. This means that incentives need to be put in place to facilitate an implementation of the necessary technology and expertise for production of food from RRM. It is also questionable whether facilitating large scale fishing is more sustainable compared to small scale fishers, who might feel more responsibility for their catch and the aquatic environment (Section 9.2.1). As long as no incentives are in place, it is unreasonable and wrong to expect any radical change. However, by joining forces to increase consideration, collaboration, and exchange of knowledge across disciplines, we are one step closer to a whitefish industry that is sustainable for the environment, for the economy and for the people. This thesis is an attempt to promote such a development.

10 Conclusion

This thesis presents a systemic and innovative approach to the Norwegian whitefish industry and the objective of improving the utilization of whitefish RRM. Laboratory experiments showed that saithe heads and backbones are suitable for bulk production of high-quality protein products for human consumption through biotechnological processing. Bioreactors can in this context be used as a step in a controlled upscaling of enzymatic hydrolysis from laboratory scale to pilot and industrial scale. UF effectively concentrated small peptides but did not increase the antioxidative activity of the product. Based on the obtained knowledge presented in this thesis and available literature, it is my opinion that the most industrially relevant application of saithe RRM is bulk production of products that could be used as protein supplement or as an ingredient in food to improve functional properties and/or extend shelf-life.

Laboratory experiments were combined with observations and interviews with fishers to identify internal and external factors that could affect the potential for improved utilization of whitefish RRM. Logistical, technological, and sociocultural factors, in addition to factors concerning the raw material itself, were identified. While the availability and seasonal stability of saithe heads and backbones could positively affect the potential for improved utilization of whitefish RRM, insufficient processing solutions, communication, management, and organization of the value chain could have a negative effect.

The work presented in this thesis does not provide a final solution, nor an all-encompassing truth, but can hopefully inspire other natural scientists to look beyond the limitation of traditional research methods and see the value in adopting and developing new methods for obtaining knowledge. Expanding our definition of valuable knowledge allows us to see that a more sustainable future cannot result from a series of individual achievement, but through cooperation between fishers, researchers, consumers, and all others involved.

11 Future aspects

The factors identified in this thesis that could affect the potential for improved utilization of whitefish RRM is merely the onset of unravelling the complexity of this objective. Future research should be conducted with the aim of identifying more factors, so that the end result is a complete map showing us the direction towards a more sustainable future. However, more work also needs to be done on adopting less traditional approaches and methods in natural science when dealing with complex problems. Problems such as obstacles to overcome in order to achieve a more sustainable future for the planet and all its living organisms.

Future research should also focus on the functionality of biotechnological processing in scales that are relevant for industrial applications: a switch from "what is possible" to "what is realistic and achievable". Research is at its best when it is explorative and idea generating, but equally important is the ability to know when it is time to move out of the laboratory where technology can contribute to a more sustainable future. Research activities over the last decades have generated extensive knowledge of the technology relating to enzymatic hydrolysis, and it is now time to put this knowledge to use. Bioactive peptides and the use of UF constitutes an exciting new path with a lot of opportunities that need further research. This research must however not lose track of the main goal, that is reducing the amount of waste, in the hunt for the most valuable compounds.

This thesis has hopefully demonstrated the value of listening to the thoughts and opinions of relevant stakeholders outside the academic sphere. I believe that similar research can greatly benefit from adopting a systemic approach, so that someday it becomes a normality rather than an exception. A new methodology toolbox has been created, and hopefully future research can contribute to its growth.

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Appendix I: Interview guide

The interview guide is organized according to the planned structure of the interview, and consists of introduction, reflection, and ending. Information provided to the participants are presented in normal font, while questions are presented in *italic*.

INTRODUCTION

General information about the PhD project, experimental work, aims and scope.

- *How old are you?*
- *What is your job title?*
- *What are your work tasks?*

REFLECTION

- *Defined as all steps from the ocean to the dinner table – what is your role in the whitefish value chain?*
- *What are your thoughts on sustainable utilization of whitefish?*
- *Are whitefish by-products/ rest raw materials valuable?*
    - *What are they composed of?*
    - *Do they have some areas of application?*
    - *Can they be used as food?*
- *Do you see any problems with the whitefish industry as operated today? If so, what can be done to improve the situation?*
- *What are your thoughts on political decisions and regulations implemented to make the utilization of whitefish more sustainable?*
- *What are your thoughts on research and technology developments as tools to make the utilization of whitefish more sustainable?*

ENDING

Information about data processing, anonymization, and need for further contact

PAPER I

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The value chain of the white fish industry in Norway: History, current status and possibilities for improvement – A review

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ABSTRACT

Inadequate utilization of available food resources constitutes a major problem regarding the growing population, the increased demand for food and the current need to reduce the global footprint exerted on the planet. Understanding value chains, identifying pressure points and room for improvement using new technological solutions, are important steps to enhance raw material utilization for the individual industries. Marine resources generated from the fish industry is an important part of the Norwegian food production and contributes about 4% to mainland gross domestic product. The most important industries are Atlantic salmon (*Salmo salar*) aquaculture, and wild capture of pelagic fish, cod (*Gadus morhua)* and saithe (*Pollachius virens)*. Processing to saleable products, mainly fillets, generates large amounts of marine rest raw material. Logistics of aquaculture production and processing allow for close to 100% utilization, while only 44% of the white fish rest raw material is being utilized. Farming of fish close to shore enable processing of rest raw material immediately after slaughter, which is paramount to avoid deterioration. Cod and Saithe are mainly caught at sea by sea trawlers and the coastal fleet, with on-board handling and transportation to shore being hurdles to overcome for processing of rest raw material to high quality products for human consumption and animal feed. This review will focus on the value chain of the white fish industry in Norway, its history, current status and possibilities of improvement to better utilize rest raw material.

Contents

1. History of marine raw material utilization

The elongated coastline of Norway has given its population good access to marine food resources, and fish has consistently been an important part of the Norwegian diet. Historians even

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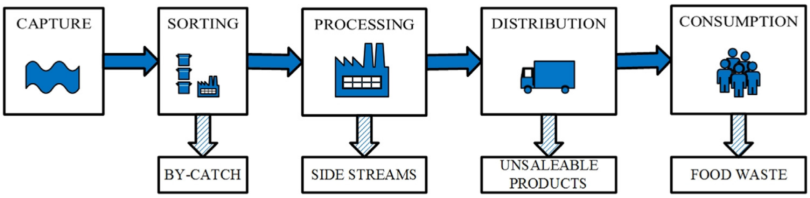

Fig. 1. The value chain of the white fish industry in Norway.

claim that herring and potatoes saved the Norwegian population from starvation and ensured its growth throughout the 19th century.

In a society scarce in food, the focus lied on utilizing as much as possible of available resources for own consumption. Norway, as many other countries at that time, experienced a growing prosperity during the last part of the 20th century, and with it an interest in marine food production aimed at the global market. This resulted in a higher product demand requiring increased efficiency and production rates, which was not necessarily consistent with optimal utilization. Changes in customer quality requirements also caused a shift in what was perceived as food, with fillets becoming the desired product from most fish species. Exceptions include roe and cod liver oil, which has a long history of use as traditional food and spread, and nutraceutical for treatment of health issues, respectively. The first recorded use of cod liver oil in Northern Europe dates back as far as 1783 (Curtis et al., 2004).

The fish industry is an important contributor to the Norwegian food production and economy, with a contribution of 3.9% to the gross domestic product (GDP) in 2017 excluding the petroleum industry (mainland GDP). Of the total contribution of 93 830 million NOK, aquaculture and wild capture generated 62 300 and 37 380 million NOK respectively (Richardsen et al., 2018). Compared to the other food industries in Norway, the seafood industry had the highest turnover value (79 535 million NOK) in 2016 and the second highest number of employments (11 280), surpassed only by the meat industry (11 408) (NIBIO, 2018). Norway is the world's second largest seafood exporter, exporting 95 % of its farmed and wild caught fish. Of the total value of all exported goods in 2017, reported to be 860 692 million NOK including petroleum, 92 241 million NOK came from fish, crustaceans, and mollusks (StatisticsNorway, 2019).

Atlantic salmon (*Salmo salar*) aquaculture, wild capture of pelagic fish and white fish are the most important contributors to the Norwegian fish industry. This review will mainly be dealing with wild capture of white fish, which is a collective term used to describe the species cod (*Gadus morhua)*, saithe (*Pollachius virens),* haddock (*Melanogrammus aeglefinus*), ling (*Molva molva*), and tusk (*Brosme brosme*). These species are exported as unprocessed, semi-processed or processed products as presented in Table 1. Cod is the biggest contributor to wild capture of white fish in terms of catch volume. Statistics Norway reported a catch of 416 994 tons of cod in 2017, accounting for 17% of the total wild capture of Norwegian fishing boats (StatisticsNorway, 2018). Of the total amount of exported white fish (244 959 tons) in 2017, cod, haddock, saithe, ling, and tusk accounted for 141 387, 50 843, 46 844, 3737, and 2148 tons respectively NorgesSjømatråd (2017).

2. The value chain of the white fish industry

According to Kaplinsky and Morris (2000), the value chain can be defined as the full range of activities required to bring a product from conception, through the different phases of production, delivery to the final customer, to disposal after use. For the white fish industry in Norway, this implies the consecutive steps from catch, through processing, arriving at retailers as saleable products or export, and finally ending up as consumed product. Huge amount of raw material is lost along the value chain, making it far from linear with respect to the fish wet weight. Raw material leaves the value chain at each consecutive step which combined result in a great loss of resources that could otherwise serve as food or other products for human and animal consumption. An overview of the value chain of wild captured white fish is illustrated in Fig. 1.

The total amount of available raw material from the fish industry in Norway for 2016 was reported to be 3.3 million ton, of which aquaculture, wild caught pelagic fish, and wild caught white fish yielded 43%, 34%, and 23% respectively (Table 2) (Richardsen et al., 2017). Processing of the raw material generated substantial amounts of rest raw material, close to 0.9 million tons for the three industries combined. For white fish, the generated rest raw material constituted over 40% of the total catch wet weight.

As is evident from Table 2, the degree of rest raw material utilization is species dependent. For aquaculture and pelagic fish, the degree of utilization is close to 100%. Wild capture of white fish generates about the same amount of rest raw material (319 000 tons) as aquaculture (400 842 tons), but for these species merely 44% is utilized. The only species where the rest raw material is utilized to a lesser extent is shellfish (25%), but the amount produced is negligible compared to white fish. White fish rest raw material is thus the fraction with the biggest potential for better utilization and value creation. This review will try to identify problems and possible solutions to improve the utilization of white fish rest raw material (Fig. 4). A complete mapping will not be possible given the information available, but the ideas presented can hopefully act as a guideline for further research on the topic in order to improve the current situation.

2.1. Harvest and processing

Atlantic salmon is the only fish species being farmed in considerable amounts in Norway. Farming of cod was also looked upon with great enthusiasm by the Norwegian aquaculture industry in the early 21st century (Rosenlund and Skretting, 2006). However, the challenges associated with the early life stages of cod, fish health and quality, adaptation of necessary equipment, and the modest filet yield compared to salmon, became too big of a challenge for cod farming to thrive in the aquaculture sector. In addition, wild capture of cod continues to be a reliable source of sufficient amount of raw material to the industry.

Unlike aquaculture, where slaughtering can be adjusted according to market demand, wild capture is dependent on a natural resource with seasonal variations in availability and composition. Aquaculture also has the benefit of being on-shore, where processing facilities generally are located in close proximity to slaughtering locations (Richardsen et al., 2017). While the common practice of the coastal fishing fleet is daily returns to shore,

Table 1
Norwegian export of unprocessed, semi-processed and processed white fish in 2017, associated amounts (tons, %) and value (billion NOK, %).
Source: Numbers obtained from NorgesSjømatråd (2017).

Exported product	Amount (tons)	Amount (% of total)	Value (billion NOK)	Value (% of total)
Frozen	184 983	42.7	5.33	34.4
Fresh/chilled	108 709	25.1	3.49	22.5
Conventional	125 693	29.0	6.41	41.4
Dried/salted/smoked	13 527	3.1	0.26	1.7

Table 2
The amount of harvested raw material (alive weight in tons), available rest raw material generated and degree of utilization, from wild capture and aquaculture in Norway 2016.
Source: Freely adopted from Richardsen et al. (2017).

Fish species	Raw material	Rest raw material	Utilized rest raw material
White fish (wild capture)	746 400	319 000	44%
Pelagic fish (wild capture)	1 090 000	177 600	100%
Aquaculture	1 394 000	400 842	91%
Shellfish	49 200	12 300	25%

harvesting by deep-sea trawlers often occur at distances days of transport from shore and land-based processing plants. This constitutes one of the most important challenges for white fish rest raw material utilization, as limiting time from capture to processing is paramount to maintain product quality (Thorkelsson et al., 2009).

Wild caught cod is an internationally desired product, exported mainly as semi-processed lower-value products (Table 1) (Trondsen, 2012). Processing of cod in Norway prior to export mainly involves freezing, salting, drying, and filleting. The share of fish raw material being processed has declined over the last years, with recent numbers indicating about 58%. The rest is exported as unprocessed whole fish. For farmed salmon, only 16% of the fish is processed, making the total export of unprocessed raw material of white, pelagic and farmed fish 72% in 2018 (NorwegianSeafoodCouncil, 2018). A lack of fish processing results in a loss of potential for value creation beneficial for the Norwegian economy, and leaves international processors and distributors with good opportunities for production of value added products (Trondsen, 2012). Fish processing abroad also entails less control regarding handling of the rest raw material from filleting etc. As will be described in the following section, fish rest raw material constitutes a source of valuable biological components that could be utilized in production of value-added products for human consumption.

Generation of rest raw material and low degree of pre-export processing are two areas of the white fish industry that could benefit from process innovation. Defined as the implementation of new or significantly improved production or delivery methods, process innovation includes changes in the equipment, software, production techniques, value chain and logistics (OECD, 2005). As described above, relocating fish processing from export destinations back to Norway can result in value creation and economic growth. It would also create new job positions for the Norwegian population. By simultaneously implementing technology enabling increased utilization of marine rest raw material, process innovation could also ensure resource protection and a more sustainable utilization. Successful implementation of enabling technology in the white fish industry will require an understanding of the value chain as such, potential hurdles in the processing line, and acceptance among both the industry and consumers. The role of qualitative analysis in generating the knowledge necessary for making improvements will be discussed in a later section.

3. Marine rest raw material

Marine rest raw material can be defined as parts of the raw material that are not the primary product of use (Richardsen et al., 2017). For fish this includes skin, scales, bones, heads, cut-offs, roe, milt and visceral components. In Norway, rest raw material can be categorized depending on handling. To be applicable for human consumption and animal feed, the rest raw material must be handled according to the hygiene regulatory framework. Rest raw material handled according to the byproduct regulatory framework is not suitable for human consumption but can still be used for animal feed if the quality is good. Low quality and risk material on the other hand, is used exclusively in production of biofuels, as fertilizers or destroyed (Richardsen et al., 2017).

By-catch and processing of fish to saleable products, mainly fillets, generates considerable amounts of rest raw material. For processing of cod, fillet products can account for as little as one-third of the fish by body weight (Falch et al., 2006). About half of the marine rest raw material generated in Norway is currently being utilized, but mainly for animal feed and other low-value products. There are several beneficial aspects associated with an increased processing to products intended for human consumption. An increase could potentially yield a five-fold value-addition seen from an economic perspective, but also contribute to a reduction of malnutrition and hunger in a global perspective (Undel et al., 2009; Rustad, 2003). Reducing the amount of food waste would also be beneficial for the environment, ensuring a more sustainable utilization of available resources. Although products for human consumption will be the focus of this review, pet food is worth mentioning as another potential product with a high marked value compared to the traditional fish meal and fish oil produced from silage. As is described in a recently published report on increased refining of seafood and rest raw materials in Norway (InnovasjonNorge, 2018), pet food is expected to increase in value due to marked forces including humanization of pets and increased focus on sustainable production from the pet owners. Some Norwegian pet food producer are operating today.

The amount of rest raw material generated from processing of marine resources in Norway range from 43% of total weight for cod to 16% for pelagic fish (Richardsen et al., 2017). As described earlier, the degree of utilization varies between species, with the majority of underutilized rest raw material originating from the white fish industry (Table 2). An approximate composition of cod and saithe rest raw material is presented in Table 3. One of the main reasons for the low degree of rest raw material utilization is the logistic and technological challenges associated with wild capture of white fish, particularly for the sea-going fleet. White fish species are primarily harvested off-shore as opposed to aquaculture harvesting located on-shore (Richardsen et al., 2017). The most important challenge regarding the quality of the rest raw material, and consequently the quality of the

Table 3
Barents sea trawler vessel average daily catch of cod and saithe, and generated rest raw material, producing 10 000 kg fillet.
Source: Freely adapted from Falch et al. (2006).

Fish species	Daily catch (kg)	Rest raw material	Total quantity	
			kg	%
Cod	29762	Viscera[a]	2 560	8.6
		Liver	1 548	5.2
		Head, backbone and trimmings	15 655	52.6
		Total rest raw material	19 762	66.4
Saithe	27 100	Viscera[a]	2 249	8.3
		Liver	1 626	6.0
		Head, backbone and trimmings	13 225	48.8
		Total rest raw material	17 100	63.1

[a]Viscera without liver.

products obtained from processing, is to limit the time from catch to processing. Fish rest raw material is a very unstable and easily perishable raw material, which makes rapid chilling, storage at low temperatures, hygiene, and short time between catch and processing crucial for utilization and production of safe products for human consumption (Thorkelsson et al., 2009).

Today, many trawlers operate with freezing units that enable frozen storage of fish from the time of capture to further processing or export. Frozen storage under appropriate conditions has proven to be efficient for storing without compromising the quality of cod muscle (Boknes et al., 2001). As temperature is one of the most important factors affecting stability, frozen storage can also contribute to prolonging the shelf life of fish (Thorkelsson et al., 2009). Freezing of rest raw material in trawlers for transport back to on-shore processing facilities could thus be one solution to improve the possibility for further processing to high quality products. Spoilage can also be reduced without freezing by protecting the rest raw material from oxygen, light and high temperatures until further processing. The development of climate chamber technology, enclosed chambers where environmental conditions can be controlled and monitored, for the purpose of research on spoilage preventative storage options is thus an interesting field with great potential for use in the fish industry. Transport of rest raw material to shore would however pose additional challenges for the industry, regarding both profitability and environmental concerns. Without economic incentives, the motivation for using space, that would otherwise be reserved for high-value fish products like filets, to transport low-value rest raw material is likely to be very low. Transport of rest raw material would also entail more trips to shore, which again results in increased fuel consumption unfavorable for the environment. In addition, fishing quotas puts pressure on the sea trawlers and coastal fleet that could amplify the perception of the economic losses that would results from rest raw material transportation.

When considering the risk of spoilage, rest raw material can be divided into two categories depending on stability. Some fractions, like head, backbones and trimmings, are relatively stable, while viscera and blood on the other hand is considered the most unstable fractions due to the higher content of endogenous enzymes (Rustad et al., 2011). Trawler sorting systems could be another solution to increase stability and facilitate production of differentiated high-value products for human consumption. Traditional sorting of cod rest raw material involves separating liver and roe from the rest of the viscera, for production of cod liver oil and roe products for human consumption respectively. Cod liver make up a considerable amount of the viscera, but only a small fraction of the total rest raw material (Table 3). Liver and roe size also vary depending on season (Falch et al., 2006). Taking this into account, sorting of liver and roe is not sufficient to ensure sustainable utilization of cod rest raw material. In the white fish (Larsen and Isaksen, 1993) and pelagic sector (Van Rijn et al., 2017), attempts have been made to develop sorting technology in the trawling process to reduce the amount of generated by-catch. On board sorting, apart from beheading, gutting, and separation of liver and roe for cod, is however not a common practice on trawlers today. Combined with freezing, improved on-board sorting technology could further contribute to maintain the quality and stability of the rest raw material up to processing. More research should thus be focused on developing such systems in the future.

An alternative to freezing would be on-board processing of rest raw material with biotechnological solutions like silage and enzymatic hydrolysis. Enzymatic hydrolysis, with optimized processing conditions, can be used to recover proteins and lipids from the rest raw material (Šližyte et al., 2005). A lot of research has been put into developing on-bard processing technology, among others a project by Nordic Wildfish involving a trawler built with a hydrolysis model system that process white fish rest raw material directly after capture (Snøfugl, 2016). While enzymatic hydrolysis with industrial enzymes is an expensive technology that yield high-value products suitable for human consumption, silage is a low-cost and simple technology that have been utilized for years to produce low-value products like fish meal and fish oil (Rustad, 2003). Despite the lower quality of the obtained products, on-board silage is an improvement to wasting fish rest raw material and is currently utilized by some Norwegian trawlers. The issues mentioned for rest raw material transport also applies to on-board processing. Successful implementation of processing technology on Norwegian trawlers would likely require economic incentives in addition to further research on development and adaptation.

4. Potential applications of marine rest raw material for human consumption

The potential application of marine rest raw material (Aspevik et al., 2017; Rustad et al., 2011) and its bioactive components (Thorkelsson et al., 2009; Hamed et al., 2015; Kim and Mendis, 2006; Kim and Wijesekara, 2010; Harnedy and FitzGerald, 2012) has been thoroughly reviewed, recently by Le Gouic et al. (2019). Marine rest raw material might be a source of essential n3 polyunsaturated fatty acids (PUFAs), high quality protein and bioactive peptides, but also essential vitamins and minerals. The main constituent vitamins and minerals of seafood, often deficient in carbohydrate rich food sources, are vitamin A and D, iodine, iron, selenium, phosphorous, magnesium and calcium (Elvevoll and James, 2001). With appropriate handling, these nutrients can be suitable for human consumption as both food and functional components (Rustad, 2003). Functional components, or functional food, are terms used to describe any ingredient or food that has a positive impact on an individual's health, physical performance, or mental function, in addition to its nutritive value (Hardy, 2000).

4.1. Proteins

Fish constitute a protein source of high nutritional value, both regarding amino acid composition and bioavailability. The amino acids arginine and tyrosine, known for their health beneficial effects, are prevalent in fish protein. Fish is also rich in lysine and threonine, amino acids that are limited in the cereal-based diet of people in developing countries (Undel et al., 2009). In addition, fish protein can act as a source of bioactive peptides

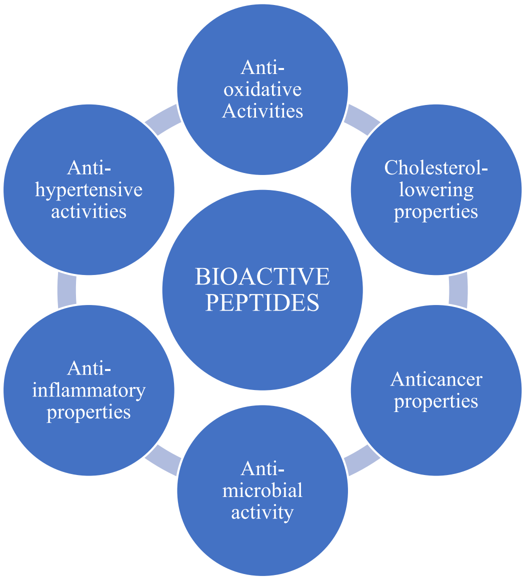

Fig. 2. Possible pharmacological effects beneficial for human health exerted by bioactive peptides derived from food and rest raw material.
Source: Freely adopted from Udenigwe and Aluko (Hartmann and Meisel, 2007).

with a number of possible health beneficial effects, including antihypertensive (Jensen and Mæhre, 2016; Ngo et al., 2016; Farvin et al., 2014; Girgih et al., 2015). An overview of the potential beneficial effects for human health exerted by bioactive peptides is presented in Fig. 2. Bioactive peptides are hydrolyzed protein with inherent functional properties beyond that of normal nutrition, and exist as an inactive part of the native protein (Hartmann and Meisel, 2007). They can be released from the native protein by in vivo (digestion) or in vitro (food processing, fermentation, enzymatic hydrolysis) proteolysis (Udenigwe and Aluko, 2012). Recovery of protein and isolation of bioactive peptides are effective means to increase utilization and value of protein rich rest raw material (Udenigwe and Aluko, 2012).

4.2. Lipids

Fish rest raw material is generally a good source of the two n3 PUFAs eicosapentaenoic acid (EPA 20:5, n3) and docosahexaenoic acid (DHA 22:6, n3). A calculation done by Falch et al. (2006) showed that production of 10 000 kg white fish fillet on average generated rest raw material containing more than 1000 kg marine lipids, of which 30% were n3 PUFAs. Consumption of marine oils is associated with several health benefits, where reduction of the risk of cardiovascular diseases (CVDs) is the most documented effect (Turner et al., 2006).

Marine lipids have a high degree of unsaturation, making them especially prone to oxidation. For PUFAs the rate of oxidation increases with the number of double bonds, making long chained n3 PUFA especially vulnerable (Maehre et al., 2015). During oxidation, primary oxidation products can break down to volatile secondary oxidation product (aldehydes and ketones) responsible for the unpleasant odor of rancid oils (Turner et al., 2006). Oxidation can consequently cause undesirable sensory changes, but also reduce the nutritional value of the oil (Sherwin, 1978). To avoid oxidation, handling and storage of the rest raw material is very important. Exposure to oxygen and high temperatures should be avoided, as these are factors known to increase oxidation rates (Damodaran et al., 2008).

4.3. Consumer acceptance

Utilization of marine rest raw material to produce food and ingredients for human consumption is dependent on consumer acceptance. When it comes to food choices in general, the most important motives are sensory appeal, health, price and convenience (Steptoe et al., 1995). Regarding fish quality, freshness is the aspect most highly valued (Trondsen, 2012). To avoid undesirable sensory properties of the product, proper handling of marine rest raw material is crucial. The literature on consumer acceptance of marine functional food is limited, but consumers are in general positive to fish and fish consumption (Honkanen et al., 2005; Olsen et al., 2007). Fish is perceived as healthy, which might give functional food of marine origin an advantage over functional food from other sources. Even though some studies show that consumers are willing to pay more for functional foods, consumers in general are very sensitive to changes in food prices, and price is therefore likely to be a barrier for consumer acceptance (Poulsen, 1999). This is especially relevant in increasing the utilization of marine rest raw material. Using biotechnological processing methods to extract and isolate functional components remains a costly step, which means that the generated products also needs to have a high price level.

5. Traceability

Improving traceability within the white fish industry could facilitate improved utilization of marine rest raw material by providing better control and monitoring of quality and storage conditions. Traceability is a comprehensive term that is used in relation to tools that enable access to essential information about the raw material from capture, along the value chain, to the final product (Donnelly and Olsen, 2012). The development within the fish industry has been characterized by globalization of trade, combined with a lack of international standards. This makes identifying the origin and history of fish a challenging task, raising safety concerns for the industry, marketers and consumers (Thompson et al., 2005). Safety concerns could in turn act as a barrier for increasing the utilization of marine rest raw material, as perceived safety is likely to be very important when considering consumer acceptance. In a case study of a Norwegian trawler, Donnelly and Olsen (2012) found that current practice involves systems for good traceability: registration and identification of boat, date, time, type of trawl, and area of trawl. Most of the information was however not automatically transferred further down the value chain as new identification codes were provided. The study also identified a lack of registration of quality data, which is crucial in order to evaluate the usability of marine rest raw material for processing to products safe for human consumption. Despite the limited mapping of traceability practices in the white fish industry, it can be assumed that many of the current problems regarding registration and information flow could be improved by implementing automatic registration systems.

5.1. Blockchain

Blockchains became known through the increased popularity of the cryptocurrency Bitcoin in 2008. Since then it has proven a successful way of conducting transactions without the need for a regulatory third party. Blockchain technology is a system that allows everyone to validate and secure every "block" in a chain, containing information about the transaction being made. Every block build on the previous block, making the system close to fail proof.

Food, agriculture and various value chains are expected to be the most promising areas of non-economical applications for blockchains (Kshetri, 2018). Regarding traceability, blockchains could provide the necessary logistics and technology for real-time tracking of product from origin to customer. Costs could be lowered by eliminating the use of middlemen, quality could be maintained throughout the value chain and food losses could be reduced. Casado-Vara et al. (2018) proposed a new model for traceability and efficiency of the agriculture value chain by implementing blockchains. The authors emphasize that blockchains would allow the different parties of the value chain to interact in a decentralized way, avoiding time-delaying intermediaries for verification, with the same functionality and trust. Implementing blockchain could also aid the transition from a linear to a sustainable circular economy. An automatic registration system using blockchains could solve many of the problems with information flow and quality data registration faced by the white fish industry, identifying the obstacles needed to overcome in order to increase utilization of marine rest raw material.

6. Qualitative analyses

Qualitative analyses constitute an interesting alternative to the more traditional quantitatively oriented research approach and could potentially yield information complementary to qualitative data regarding research aimed at increasing utilization of marine rest raw material. For the white fish industry in Norway, qualitative analysis could be a useful tool to identify and understand the underlaying mechanisms of current practice and the low degree of utilization. Qualitative research data can thus provide valuable information about opportunities and hurdles for implementing appropriate technology and new practices. An article by Jentoft (2006) highlights the benefits of adopting a qualitative approach when conducting research on fishery science and management. Combining quantitative research with the experience-based knowledge of individual contributors of the fish industry, obtained by qualitative research methods, could be highly beneficial for fishery science and management development. The importance of qualitative research in fishery management is also emphasized in an article by Barclay et al. (2017). Their study of fisheries in Australia and associated islands resulted in an improved knowledge base identifying new issues that had not been identified under traditional research approaches. Both authors conclude that resource management, when seen as a result of a combination of regulations, practicalities, traditions, attitudes, and values, could benefit from qualitative research.

Somewhat opposed to quantitative analyses, a qualitative approach is used to explore and inductively understand (find general context in) a defined environment (case) based on empirical data (individual observations) (Tjora, 2018b). As presented in Fig. 3, inductive research moves from empirical data to theory while deductive research move from theory to empirical data. Similarly, technology development can be viewed as an inductive process, moving from the industrial observations to laboratory research. In the same line of thinking, technology implementation could be understood as a deductive process moving from the laboratory to the industry. Data and knowledge gathered from the white fish industry through qualitative analysis could consequently contribute to successful technology research and development.

Two ways of generating qualitative analytical data from the white fish industry is through observational studies and in-depth interviews. Observational studies are ethnographic in nature,

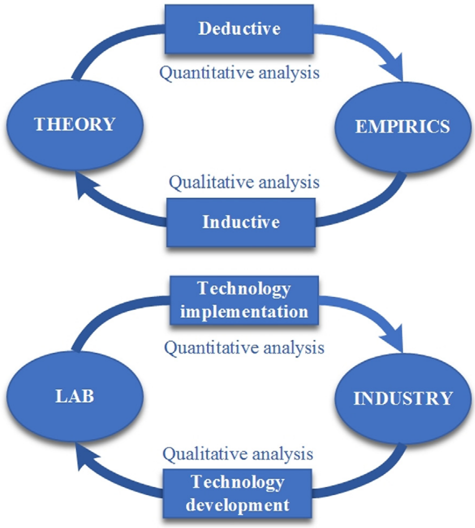

Fig. 3. Illustration of the analytic process of quantitative analysis, moving from theory to empirics, and qualitative analysis, moving from empirics to theory. Technology development viewed as a qualitative analytical process and technology implementation as a quantitative analytical process.

meaning that the researcher participates as an open or hidden part of the everyday activities related to a specific case for a given period of time. The researcher collects all available data that could help enlighten the research topic, including description of activities, communication, and answers to questions asked by the researcher (Hammersley and Atkinson, 2007). In-depth interviews are a semi-structured way of obtaining data, with the goal of obtaining a relatively free conversation with the person being interviewed. The conversation should evolve around topics pre-determined by the researcher, but the questions are not closed as in typical surveys. In-depth interviews are thus a useful tool in highlight opinions, attitudes and experiences among the participants (Tjora, 2018a). Regardless of method, qualitative analysis is a promising approach to generate knowledge that could contribute to a development towards a more sustainable consumption of white fish raw material in Norway.

7. Concluding remarks

The Norwegian fish industry has a long tradition and continues to be an important contributor to the food production and economy in Norway. Aquaculture of salmon, and wild capture of pelagic and white fish, constitutes the most important contributors. Catch and processing of fish generates huge amounts of rest raw material, which is utilized to different degrees depending on fish species. The lowest degree of utilization is found for white fish rest raw material, while most of what is utilized goes to production of low-value products like animal feed. This can thus be viewed as a twofold problem; since the coastal fleet lands most of its raw material, the main improvement here will be increasing the value of the products by aiming at human consumption rather than animal feed. Marine rest raw material is a source of valuable components like lipids, proteins, vitamins, and minerals, which could act as a source of both food and functional components. The seagoing fleet faces different challenges, as described in Section 3. A general increase in utilization will be of a larger importance for this part of the industry.

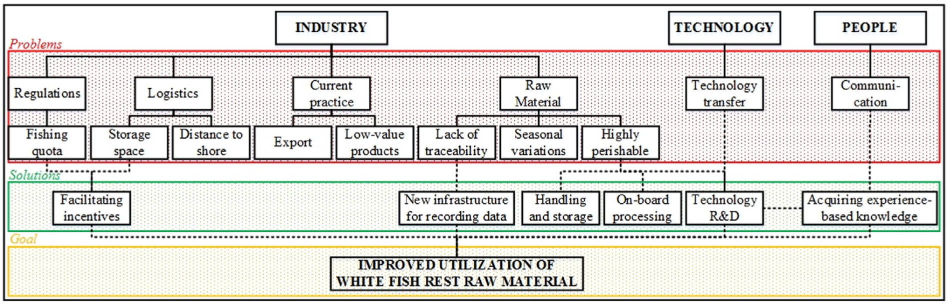

Fig. 4. Overview of the highlighted problems and suggested solutions to improve utilization of white fish rest raw material for the industry, technology and people. The illustration shows that more research is needed for a complete mapping of the potential problems, a criterium to further identify and develop appropriate solutions, and can thus potentially act as a guideline for a more systemic approach in future work.

According to a design thinking method, the best space of innovation is located in the midst between industrial (business) needs, people needs and the appropriate technology (Curedale, 2018). Our attempt to illustrate this is presented in Fig. 4. This review has identified several problems of the white fish industry that can act as hurdles to improve rest raw material utilization, both regarding regulations, logistics, raw material and current practices. However, more research and development need to be conducted in order to find the most appropriate technological solutions. Further, the technology needs to be transferred from research facilities to the industry for implementation, which in itself constitutes a problem. Section 6 briefly touched upon qualitative analyses, that are at core of design thinking, as a tool to better understand people needs. Obtaining a holistic understanding of the white fish industry, by accessing experienced based knowledge and individual needs through qualitative analysis, is in the authors opinion a highly interesting approach which should be examined in future research. Further, such knowledge could be very useful in order to develop the appropriate technology. Qualitative analysis could also be useful in order to obtain a better understanding of the factors contributing to a higher degree of utilization for aquaculture and the pelagic fish industry. The overview depicted in Fig. 4 clearly shows that a lot of research remains in order to identify and understand all problems and find the best solutions to achieve the desired goal: improved utilization of white fish rest raw material.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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PAPER II

This paper is awaiting publication and is not included in NTNU Open

PAPER III

catalysts

Article

Ultrafiltration of Saithe (*Pollachius virens*) Protein Hydrolysates and Its Effect on Antioxidative Activity

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Abstract: The whitefish industry generates a huge amount of rest raw material, which is currently wasted or underutilized in the production of low-value products such as animal feed. While fish muscle is the primary product of use for human consumption, rest raw material has great potential as a source of protein and bioactive peptides for the production of food ingredients and nutraceuticals. Enzymatic hydrolysis is a biotechnological processing method that can be used to extract protein from fish rest raw material into a protein hydrolysate. This study aimed at investigating the functionality of ultrafiltration as an industrial processing method and its effect on the bioactivity of protein hydrolysates. Protein hydrolysates were produced by enzymatic hydrolysis of saithe (*Pollachius virens*) head and backbone caught at two separate occasions to investigate the effect of seasonal variations. Ultrafiltration effectively concentrated larger peptides (>4 kDa) and smaller peptides (<4 kDa) in separate fractions, with a protein yield of 31% in the fraction <4 kDa. The unfiltered hydrolysate was found to have a higher antioxidative activity compared to the <4 kDa fraction in ABTS, FRAP, and ORAC assays. These results indicate that ultrafiltration does not effectively increase bioactivity by concentrating small peptides and that bioactivity is dependent on several properties, including interaction with larger peptides.

Keywords: fish rest raw material; protein; bioactive peptides; antioxidative peptides; biotechnological processing; enzymatic hydrolysis; ultrafiltration

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1. Introduction

It is well documented that fish is an excellent source of several health-beneficial nutritional components, such as polyunsaturated fatty acids and protein with a well-balanced amino acid composition [1]. The consumption of pure muscle in the form of filet or processed fish products is the most common way of including fish protein as part of the human diet. In addition, niche products consisting of heads, roe, and liver are consumed in some countries, but fish rest raw material (RRM) is predominantly an underutilized resource. RRM can be defined as the parts of the fish that are not the primary product of use, which in most cases is the filet; RRM includes heads, backbones, and viscera [2]. Fish heads and backbones have a high protein content of 15–20% [3–6]. However, RRM needs to be processed in order to be suitable as food or food ingredients for human consumption due to the nature of its composition and appearance. Biotechnological processing by enzymatic hydrolysis can be used to extract proteins from the RRM by the hydrolytic activity of endogenous or commercial enzymes. The enzymatic activity results in solubilization of the proteins, which then are extracted as the main product of enzymatic hydrolysis: the fish protein hydrolysate (FPH). Hence, the processing of fish RRM enables a better utilization of fish as a food resource, which is beneficial from both sustainability and a bioeconomical perspectives [7,8]. In addition to being an excellent source of amino acids for human metabolism, fish RRM can be a source of bioactive peptides that are inactive as part of the native protein but can be released through a hydrolytic process [9]. Bioactive peptides are small molecules, ranging from three to 20 amino acids, with inherent health-beneficial properties beyond that of normal nutrition [10,11]. Several studies have identified bioactive peptides in whitefish [12,13]. Cod protein hydrolysates have been found to possess both antioxidative and blood pressure reducing activities in vitro [14–18], while in vivo animal studies have been less conclusive [19].

Reactive oxygen species (ROS) are generated continuously and unavoidably through cellular respiration in our body and during lipid oxidation in foods and are therefore a cause of great concern both regarding human health and food stability. Oxidative stress, defined as an unbalance between ROS and endogenous antioxidants, has been linked to several adverse health effects and human diseases, and lipid oxidation in food can cause a reduction of shelf-life and nutritional value as well as unwanted changes to odor and texture [20,21]. Antioxidants are molecules capable of reducing oxidative stress and lipid oxidation in food by inhibiting oxidation [22]. These molecules are naturally occurring in living tissue, and both natural and synthetic antioxidants are used as additives in various foods. However, commonly used synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have received negative attention due to the toxicity of these in high concentrations [22,23]. Thus, there is a strong interest in finding natural alternatives of antioxidants for food purposes but also for the use as nutraceuticals [20]. For any substance to act as an antioxidant, it must be able to neutralize ROS or other prooxidative components, thereby preventing them from causing oxidative damage in food systems or human tissue. This can be achieved by several mechanisms, including the scavenging of ROS and metal chelation [24]. In turn, ROS can be neutralized by hydrogen donation or electron transfer, which are abilities measured by the oxygen radical absorbance capacity (ORAC) and 2.2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)/ferric-reducing antioxidant power (FRAP) assays, respectively. A combination of assays is preferred to analyze antioxidative activity, as the individual assays are limited by their specific conditions and the mechanisms they assess [22,24].

The Norwegian whitefish industry generates approximately 300,000 tons of RRM each year [2]. Improving the utilization of RRM is fundamental for making fisheries more sustainable to meet the UN Sustainable Development Goals (SDGs). Sustainable fisheries are crucial to improve the utilization of available food resources (SDG 12), reduce hunger and malnutrition by making available more healthy food (SDG 2), and prevent climate change (SDG 13) [25]. This study aimed at investigating the possibility of extracting and concentrating peptides from saithe (*P. virens*) RRM by enzymatic hydrolysis and membrane ultrafiltration (UF). UF is a processing and refinement method that can be used to concentrate low molecular weight peptides of a hydrolysate, which could potentially increase the bioactivity of this fraction [26–28]. Head and backbone from saithe caught at two separate occasions, to account for seasonal variation in spawning, were processed by enzymatic hydrolysis in bioreactors to extract proteins. The resulting saithe protein hydrolysates (SPH) were further fractionated using UF with a 4 kDa molecular weight cut-off (MWCO) membrane to concentrate small peptides. The entire processing was conducted in pilot scale to simulate an industrial process. As this study is part of a larger experimental design aiming to increase the utilization of saithe RRM, all experiments were conducted with a continuous focus on the adaptability of the process to industrial processing lines. The potential for implementing enzymatic hydrolysis in the Norwegian whitefish industry was discussed by Hjellnes et al. [29]. UF applied in an industrial setting would entail extra cost and logistical challenges, which means that the value of the processing product must be high to justify the cost–benefit balance. To the authors' knowledge, few studies have investigated the effect of membrane filtration on the bioactivity of whitefish hydrolysates [14–17], and most studies have focused on cod. Therefore, an investigation of intraspecies similarities between cod and saithe, as well as the potential effect of spawning on bioactivity, was of interest in this study.

2. Results and Discussion

2.1. Membrane Filtration and Protein Flow

Prior to filtration with 4 kDa molecular weight cut-off membrane (MWCO), 5 grams of SPH was dissolved in 500 mL of distilled water and filtrated through a ceramic membrane with 150 kDa MWCO in order to remove high molecular weight peptides and unwanted compounds such as lipids. Such compounds could otherwise cause clogging and the formation of a dynamic cake on membranes with a lower MWCO, reducing the filtration efficiency [30]. The initial filtration distributed the protein content of SPH in a retentate (R150) and a permeate (P150), containing peptides larger and smaller than 150 kDa, respectively. Ultrafiltration (UF) was subsequently performed on P150 using PESH membranes with a MWCO of 4 kDa, creating a new retentate (R4, >4kDa) and permeate (P4, <4kDa).

UF of the eight SPH from enzymatic hydrolysis of saithe heads (H) and backbone (B), in two bioreactors (I, II), from October (HOI, HOII, BOI, BOII) and January (HJI, HJII, BJI, BJII) resulted in a mass distribution presented in Figure 1. Filtration over 150 kDa MWCO ceramic membranes resulted in 1.42 ± 0.08 g SPH in R150 and 2.78 ± 0.05 g SPH in P150, which equals a protein yield of 56% in P150. The subsequent UF over 4 kDa cut-off PESH membranes resulted in 1.08 ± 0.08 g SPH in R4 and 1.56 ± 0.16 g in P4, which is equivalent to a protein yield of 31% of the initial 5 g of SPH in P4. The total protein loss from both filtrations was 19%.

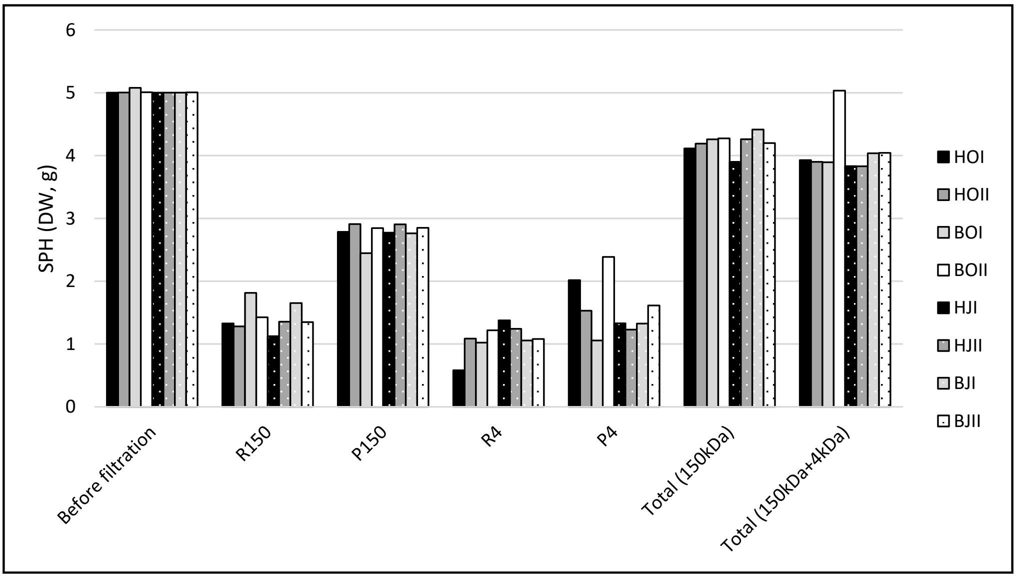

Figure 1. Mass distribution (n = 1) of saithe protein hydrolysates (SPH) before filtration and after filtration with 150 kDa cut-off ceramic membrane (retentate: R150, permeate: P150), and subsequently 4 kDa cut-off PESH membranes (retentate: R4, permeate: P4). Total mass of R150 and P150 (Total (150kDa)) and total mass of R150, R4, and P4 (Total (150 kDa + 4 kDa) is included. SPH were obtained from the enzymatic hydrolysis of heads (H) and backbones (B), in two bioreactors (I, II), from saithe caught in October 2019 (HOI, HOII, BOI, BOII) and January 2020 (HJI, HJII, BJI, BJII).

Based on an initial protein content of 16.3% and 19.2% in saithe head and backbone, respectively [3], enzymatic hydrolysis followed by UF over 150 kDa and 4 kDa MWCO membranes would give a processing protein flow, as presented in Figure 2.

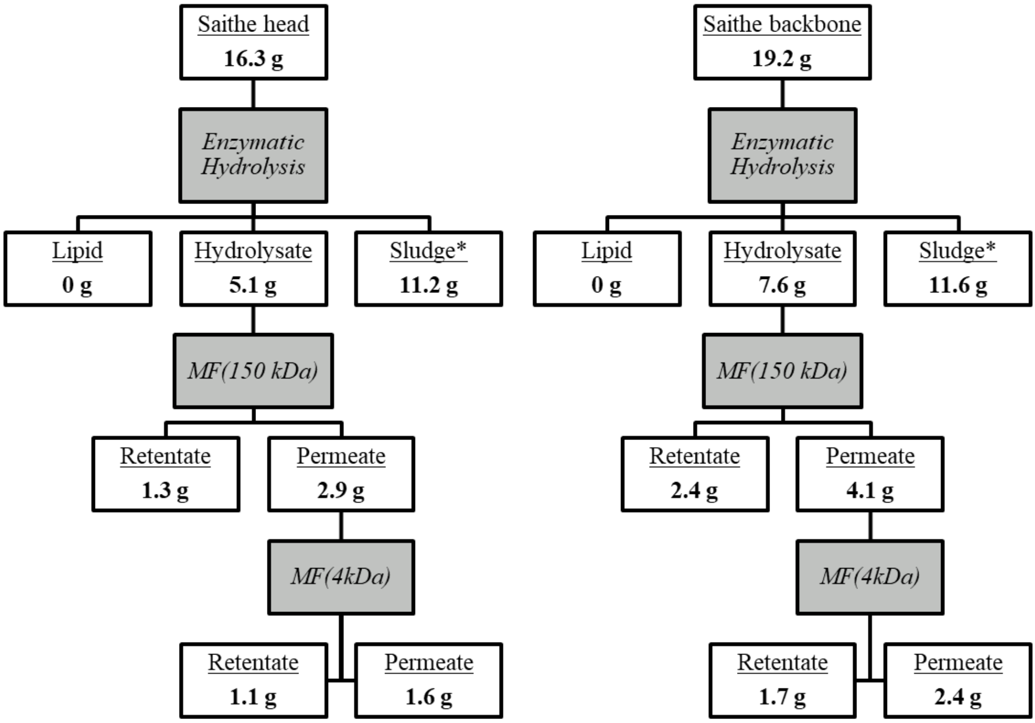

Figure 2. Protein flow (g) from raw material to product after processing of 100 g minced saithe heads (left) and backbones (right) by enzymatic hydrolysis [3] and filtration with a 150 kDa cut-off ceramic membrane followed by a 4 kDa cut-off PESH membrane. *Calculated value.

The processing of 100 g minced saithe heads and backbone yielded 5.1 g and 7.6 g protein in the hydrolysate, while 1.6 g and 2.4 g would end up in the P4, respectively. In an industrial processing line, where the goal is to obtain a pure protein fraction from fish RRM and isolating peptides with high bioactivity, SPH and P4 would be the main products of interest. This means that a large fraction of RRM protein would end up in the less regarded processing products. For the processing to be beneficial from an economic point of view, P4 would have to generate high incomes as specialized products for human consumption. However, for the processing to be sustainable, it would also be very important to find areas of application for the sludge, R150, and R4. The sludge fraction could be used for gelatin extraction and animal feed production [31,32], while the functional characteristics of the larger peptides in R150 and R4 could make them suitable as food ingredients with water-binding, emulsifying, and gelling properties [33]. It is also worth noting that it is not common practice to report protein yield of UF processing, which in the authors' opinion is a highly relevant factor in evaluating the potential for industrialization.

2.2. Amino Acid Composition

The amino acid composition of RRM and SPH from enzymatic hydrolysis of heads (HOI, HOII) and backbones (BOI, BOII) from saithe captured in October 2019 and heads (HJI, HJII) and backbones (BJI, BJII) from saithe captured in January 2020 are presented in Table 1. Glu (7.73 ± 1.75–17.13 ± 1.06 g/100 g protein), Gly/Arg (5.03 ± 0.59–17.04 ± 0.26 g/100 g protein), and Asp (5.34 ± 0.59–11.57 ± 0.73 g/100 g protein) were found to be the dominating amino acids in both RRM and SPH. Fish protein is known to have a high content of the essential amino acids Lys and Leu [13], which was also confirmed by this study, where the Lys and Leu contents were found to be 5.03 ± 0.54–9.07 ± 0.59 g/100 g protein and 3.44 ± 0.38 g/100 g protein, respectively. These results are in correspondence with Farvin et al. [15], who found Gly, Glu, Lys, and Ala to be the predominant amino acids in commercial cod protein hydrolysates; Jensen et al. [34], who found Glu, Asp, Ala, Leu, and Lys to be the dominating amino acids of cod muscle; Girgih et al. [14], who found Glu/Gln, Asn/Asp, Arg, Lys, and Leu to be the dominating amino acids in cod backbone hydrolysate >1kDa; and Remme and Austnes [6], who found Asp, Glu, and Gly to be the dominating amino acids in cod head hydrolysates.

Table 1. Total amino acid composition (g/100 g protein) in RRM and SPH obtained from enzymatic hydrolysis of heads (H) and backbones (B) in two bioreactors (I, II) from saithe caught in October 2019 (HOI, HOII, BOI, BOII) and January 2020 (HJI, HJII, BJI, BJII) ($\bar{x}$ ± SEM, n = 3). Essential [35] and hydrophobic [36] amino acids are indicated by e and h, respectively.

BOI	BOII	HJI	HJII	BJI	BJII
5.34 ± 0.59	5.45 ± 1.22	7.53 ± 0.36	8.18 ± 0.15	5.91 ± 1.59	6.08 ± 0.59
7.56 ± 0.85	7.73 ± 1.75	11.48 ± 0.59	12.35 ± 0.26	8.86 ± 2.45	8.93 ± 0.91
0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.02 ± 0.02
0.81 ± 0.10	0.52 ± 0.27	0.79 ± 0.08	1.07 ± 0.09	0.69 ± 0.31	0.80 ± 0.08
2.35 ± 0.26	2.30 ± 0.53	4.32 ± 0.23	4.49 ± 0.07	2.56 ± 0.80	2.82 ± 0.36
0.20 ± 0.05	0.14 ± 0.03	0.15 ± 0.03	0.17 ± 0.01	0.19 ± 0.13	0.16 ± 0.05
5.03 ± 0.59	5.11 ± 1.20	13.00 ± 0.63	12.70 ± 0.27	6.02 ± 1.89	5.87 ± 0.61
2.31 ± 0.28	2.34 ± 0.54	3.59 ± 0.39	4.03 ± 0.06	2.63 ± 0.81	2.61 ± 0.25
3.61 ± 0.40	3.71 ± 0.83	6.26 ± 0.29	6.53 ± 0.15	4.06 ± 1.19	4.04 ± 0.42
0.63 ± 0.07	0.37 ± 0.23	1.16 ± 0.07	1.14 ± 0.23	0.28 ± 0.12	0.52 ± 0.07
1.38 ± 0.15	1.24 ± 0.35	2.35 ± 0.00	2.53 ± 0.07	1.27 ± 0.45	1.56 ± 0.15
2.23 ± 0.25	2.30 ± 0.52	3.22 ± 0.12	3.51 ± 0.08	2.44 ± 0.61	2.48 ± 0.23
1.49 ± 0.17	1.39 ± 0.30	2.52 ± 0.16	2.71 ± 0.11	1.56 ± 0.50	1.79 ± 0.18
1.73 ± 0.19	1.74 ± 0.39	2.22 ± 0.13	2.44 ± 0.04	1.90 ± 0.48	1.99 ± 0.19
3.44 ± 0.38	3.51 ± 0.78	4.39 ± 0.23	4.89 ± 0.09	3.75 ± 0.96	3.91 ± 0.37
5.03 ± 0.54	5.16 ± 1.16	5.99 ± 0.28	6.57 ± 0.13	5.64 ± 1.57	5.95 ± 0.56
1.31	1.32	1.85	2.05	1.44	1.53

AA	Head October (RRM)	Backbone October (RRM)	Head January (RRM)	Backbone January (RRM)	HOI	HOII
Asp[h]	7.88 ± 0.31	11.57 ± 0.73	10.01 ± 0.13	9.63 ± 0.49	6.68 ± 0.10	8.18 ± 0.32
Glu	11.98 ± 0.51	17.13 ± 1.16	15.30 ± 0.18	14.47 ± 0.89	9.78 ± 0.18	12.19 ± 0.43
Asn	0.00 ± 0.00	0.11 ± 0.01	0.00 ± 0.00	0.04 ± 0.03	0.03 ± 0.02	0.00 ± 0.00
His[e]	1.43 ± 0.07	0.13 ± 0.04	2.00 ± 0.04	1.23 ± 0.57	0.42 ± 0.14	0.85 ± 0.04
Ser	4.38 ± 0.21	4.40 ± 0.30	5.86 ± 0.07	4.12 ± 0.44	3.71 ± 0.28	4.98 ± 0.24
Gln	0.20 ± 0.04	0.18 ± 0.05	0.31 ± 0.01	0.25 ± 0.08	0.12 ± 0.02	0.22 ± 0.00
Gly/Arg[h]	11.71 ± 0.70	8.33 ± 0.34	17.04 ± 0.26	7.99 ± 0.63	11.01 ± 0.37	14.27 ± 0.53
Thr[e]	2.55 ± 1.28	5.28 ± 0.37	5.26 ± 0.06	4.44 ± 0.30	3.31 ± 0.15	4.12 ± 0.19
Ala	6.30 ± 0.32	7.06 ± 0.35	8.58 ± 0.13	5.97 ± 0.30	5.52 ± 0.07	6.82 ± 0.34
Tyr[ce]	2.20 ± 0.15	0.02 ± 0.00	2.52 ± 0.06	1.75 ± 0.86	0.50 ± 0.47	1.84 ± 0.03
Met[e,h]	2.61 ± 0.09	2.92 ± 0.23	3.25 ± 0.05	2.84 ± 0.45	1.93 ± 0.17	2.59 ± 0.06
Val[e,h]	3.46 ± 0.13	5.18 ± 0.30	4.25 ± 0.05	4.20 ± 0.22	2.94 ± 0.09	3.63 ± 0.15
Phe[h]	3.23 ± 0.12	3.27 ± 0.30	3.83 ± 0.05	3.38 ± 0.54	2.10 ± 0.22	2.89 ± 0.11
Ile[e,h]	2.59 ± 0.09	4.69 ± 0.33	3.25 ± 0.05	3.89 ± 0.22	2.03 ± 0.05	2.54 ± 0.10
Leu[e,h]	4.81 ± 0.17	8.57 ± 0.57	5.86 ± 0.02	7.16 ± 0.53	3.99 ± 0.07	4.90 ± 0.18
Lys[e]	5.97 ± 0.21	9.07 ± 0.59	7.15 ± 0.14	7.88 ± 0.73	5.13 ± 0.10	6.47 ± 0.26
PER*	1.93	3.04	2.55	2.62	1.62	2.08

Spawning did not seem to have a significant effect on the amino acid composition of RRM nor SPH. However, backbone RRM was found to have a significantly higher content of Asp ($F(1,10) = 14.03$, $p < 0.05$), Glu ($F(1,10) = 6.06$, $p < 0.05$), Val ($F(1,10) = 9.04$, $p < 0.05$), Ile ($F(1,10) = 25.84$, $p < 0.05$), Leu ($F(1,10) = 26.14$, $p < 0.05$), and Lys ($F(1,10) = 13.67$, $p < 0.05$), and a significantly lower content of His ($F(1,10) = 7.28$, $p < 0.05$) and Gly/Arg ($F(1,10) = 15.37$, $p < 0.05$), compared to head RRM. Most amino acids were also found to be prevalent in higher amounts in RRM compared to SPH. The largest difference between head RRM and backbone RRM was found for Ile and Leu, which is interesting from a nutritional perspective considering that both of there are essential hydrophobic amino acids [35,36]. A product higher in essential amino acids might be more attractive both as a food ingredient and a nutraceutical.

However, the difference in amino acid composition between head RRM and backbone RRM was not reflected in their respective SPH. The only detectable trend was SPH from backbone having a significantly lower content of Gly/Arg ($F(1,22) = 24.09$, $p < 0.05$) compared to SPH from head. For the remaining amino acids, equal or higher amounts were found in SPH from head compared to SPH from backbone. However, the protein yield in SPH from backbone after enzymatic hydrolysis was found to be higher than in SPH from head [3]. This might indicate that hydrophobic amino acids, of which Ile, Leu, and Val were detected in higher amounts in backbone RRM, are harder to extract in the water-soluble phase during enzymatic hydrolysis. It is also evident from Table 1 that there are variations between the individual hydrolysates, indicating that it can be difficult to achieve a standardized processing outcome, which again might mask potential differences between SPH from head and backbone.

Although the amount of Trp, Cys, and Pro could not be detected due to the analytical method used in this study, several of the other essential and conditionally essential amino acids were prevalent in RRM and SPH. This is especially relevant when considering the nutritional quality of a protein product. The protein efficiency ratio (PER) test is a method used to evaluate the quality of a protein source based on the weight gain of rats when fed a diet consisting of 10% of the evaluated protein [37]. However, calculations on relative quantities of specific amino acids, yielding a PER value, can be used as an estimator for protein quality. The calculated PER values for RRM (1.93–3.04) and SPH (1.31–1.53) are presented in Table 1. These results correspond to those of Šližytė et al. [37], who found PER values of 2.99 for cod muscle and 1.60–2.27 for hydrolysates from hydrolysis of cod backbone and viscera under similar conditions using Flavorzyme and Neutrase. PER values were found to be significantly ($T(9) = 3.345$, $p =0 .05$) higher for RRM compared to SPH, indicating that several essential amino acids end up in the sludge. This further substantiates the importance of investigating the possibility of using the sludge fraction as a source of nutrition as well. The sludge was found to be the fraction where the majority of the RRM dry matter ended after enzymatic hydrolysis [3]. Thus, finding areas of application is important in order to minimize the amount of processing waste while simultaneously maximizing the value addition of the rest raw material.

The amount of His was found to be 0.42 ± 0.14–1.07 ± 0.09 g/100 g protein in SPH. In addition to being an essential amino acid, His has been associated with antioxidative properties due to the ability of its imidazole to form complexes with several metal ions and scavenge ROS [38,39]. The metal ion-chelating abilities of His is, among other physiological functions, important for the binding of iron in hemoglobin and myoglobin [38]. Trp and Gly, but also Cys, Ser (2.30 ± 0.53–4.98 ± 0.24g/100 g protein in SPH), Lys (5.03 ± 0.54–6.57 ± 0.13 g/100 g protein in SPH), and Pro, have been shown to potentially inhibit oxidation [40]. Despite a relatively low His content, SPH are high in Gly/Arg (5.03 ± 0.59–14.27 ± 0.53 g/100 g protein), Ser, and Lys and might thus be a source of bioactive peptides with antioxidative properties. Based on the higher content of Gly/Arg, SPH from head is likely to have better antioxidative properties compared to SPH from backbone. However, the antioxidative activity in peptides is not solely dependent on the specific amino acid present but also the sequence of their arrangement and the overall peptide composition

[21,41]. The overall hydrophobicity is also important for bioactive peptides to be able to exert their antioxidative activity by interacting with lipid systems in both our body and in foods [39]. Several hydrophobic amino acids were present in SPH, including Val (2.23 ± 0.25–3.63 ± 0.15 g/100 g protein) and Leu (3.44 ± 0.38–4.90 ± 0.18 g/100 g protein). However, Leu was found to be one of the most prevalent free amino acids in SPH, which might negatively affect its contribution to antioxidative activity and reduce bioavailability [42]. In general, the amount of free amino acids in SPH was low, ranging from 42.78 to 69.50 mg/g. This was expected, considering that both enzymes used for the enzymatic hydrolysis were endopeptidases, the low DH (14.8–18.1%) of SPH, and that head and backbone are RRM fractions with low endogenous enzyme activity [3].

2.3. Molecular Weight Distribution

The molecular weight distribution of SPH and P4 from saithe head and backbone are presented in Figures 3–6.

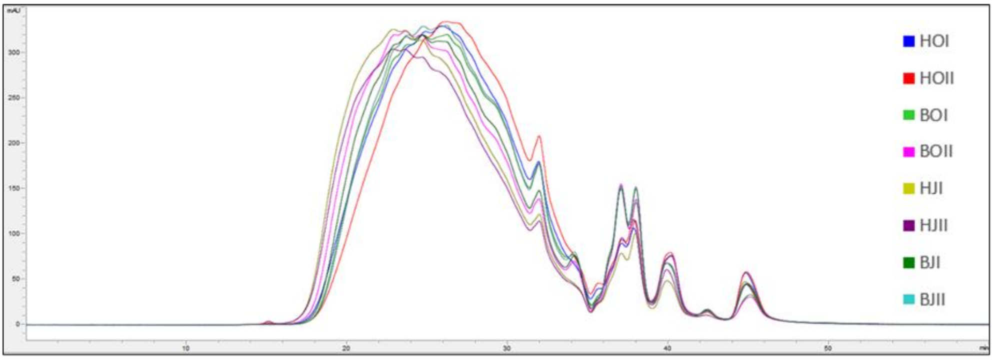

Figure 3. Chromatograms (mAU/min) from analysis of molecular weight distribution ($\bar{x}$ ± SEM, n = 2) of saithe protein hydrolysates obtained from enzymatic hydrolysis of heads (H) and backbones (B), in two bioreactors (I, II), from saithe caught in October 2019 (HOI (blue), HOII (red), BOI (green), BOII (pink)) and January 2020 (HJI (beige), HJII (purple), BJI (dark green), BJII (turquoise)).

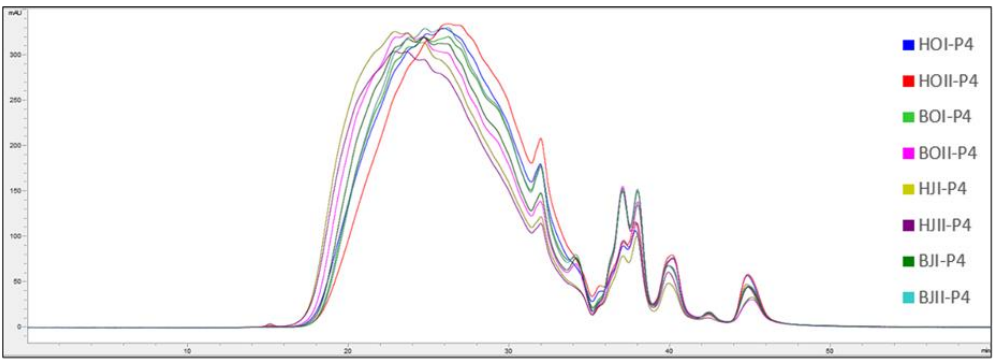

Figure 4. Chromatograms (mAU/min) from analysis of molecular weight distribution ($\bar{x}$ ± SEM, n = 2) of permeates (P4) after ultrafiltration (<4kDa) of saithe protein hydrolysates obtained from enzymatic hydrolysis of heads (H) and backbones (B), in two bioreactors (I, II), from saithe caught in October 2019 (HOI-P4 (blue), HOII-P4 (red), BOI-P4 (green), BOII-P4 (pink)) and January 2020 (HJI-P4 (beige), HJII-P4 (purple), BJI-P4 (dark green), BJII-P4 (turquoise)).

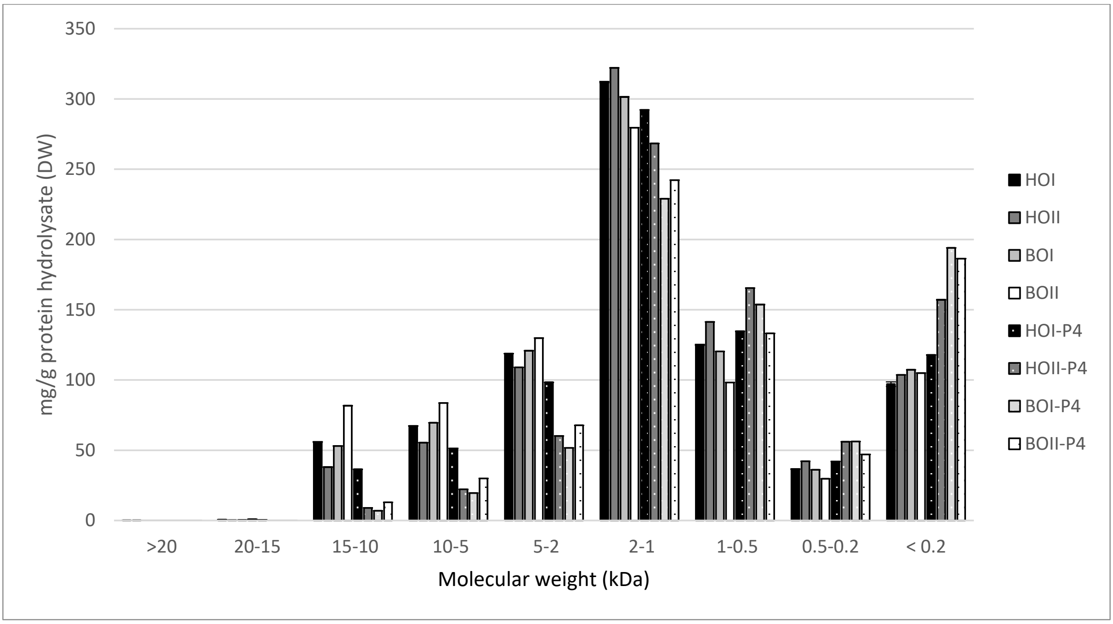

Figure 5. Molecular weight distribution (x̄ ± SEM, n = 2) of saithe protein hydrolysates (SPH) (HOI, HOII, BOII, BOII) and their corresponding permeates (HOI-P4, HOII-P4, BOI-P4, BOII-P4) after ultrafiltration (<4kDa). SPH were obtained from enzymatic hydrolysis of heads (H) and backbones (B), in two bioreactors (I, II), from saithe caught in October 2019 (HOI, HOII, BOI, BOII).

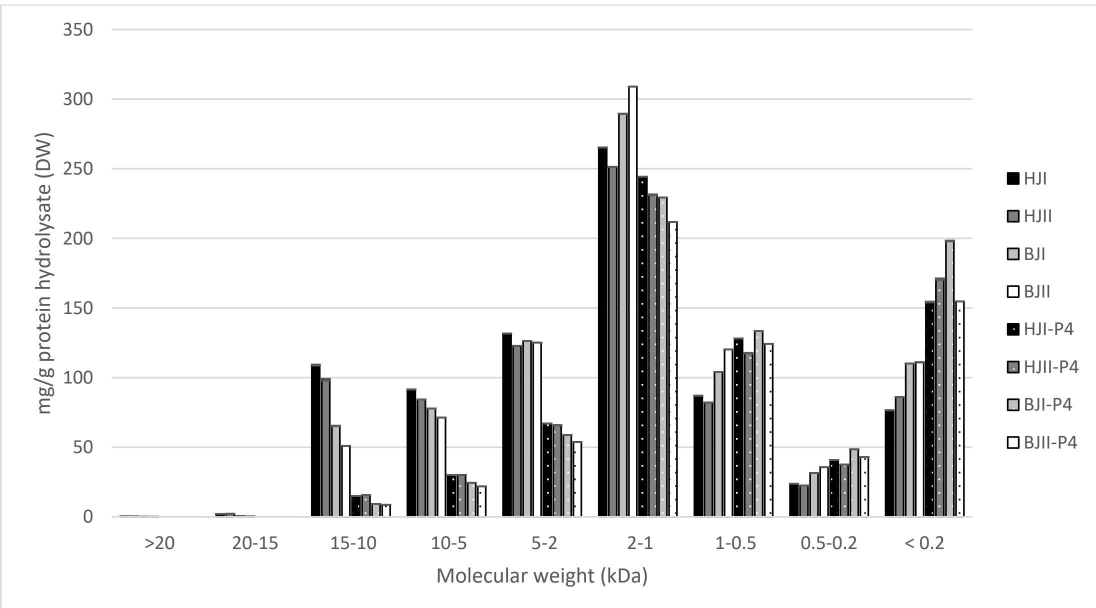

Figure 6. Molecular weight distribution (x̄ ± SEM, n = 2) of saithe protein hydrolysates (SPH) (HJI, HJII, BJII, BJII) and their corresponding permeates (HJI-P4, HJII-P4, BJI-P4, BJII-P4) after ultrafiltration (<4kDa). SPH were obtained from enzymatic hydrolysis of heads (H) and backbones (B), in two bioreactors (I, II), from saithe caught in January 2020 (HJI, HJII, BJI, BJII).

Spawning did not affect the molecular weight distribution of SPH and P4, and no difference was observed between SPH and P4 produced from head RRM compared to backbone RRM. SPH were found to have a significantly higher content of peptides in the size range 15–10 kDa, 10–5 kDa, 5–2 kDa, and 2–1 kDa, while P4 were found to have a significantly higher content of peptides in the size range 1–0.5 kDa, 0.5–0.2 kDa, and <0.2 kDa. The largest difference was observed for the size ranges 10–5 kDa ($T(30) = 12.58$, $p < 0.05$) and 5–2 kDa ($T(30) = 14.71$, $p < 0.05$), where the SPH average was found to be 75.08 mg/g and 123.04 mg/g, respectively. P4 were found to have on average 28.69 mg/g peptides in the size range 10–5 kDa and 65.44 mg/g peptides in the size range 5–2 kDa.

These results indicate that peptides >5 kDa to a large degree have been concentrated in R4 after UF, and that the majority of peptides in P4 have a molecular weight <4 kDa. However, the MWCO at 4 kDa is not clear cut, as is evident from the presence of peptides with molecular size 5–15 kDa in P4. This lack of a sharp separation of peptides during UF has also been demonstrated in other studies [15,16]. Chabeaud et al. [43,44] suggested potential fouling, lowered flux and insufficient membrane cleaning to be reasons for the poor separation of peptides. In their studies on UF of fish protein hydrolysates, they found that peptides <0.5 kDa were better separated at lower concentrations of saithe protein hydrolysate (30 g/L vs. 150 g/L) and lower pressure (1 bar vs. 5 bar). Thus, it is likely that parameters of UF can be adjusted in order to improve size-based separation of peptides.

Of the total peptide content of SPH, 23.8–35.6% were found to have a molecular size <1 kDa. Combined with a relatively low DH and the low content of free amino acids, these results indicate that SPH contains considerable amounts of larger peptides [3]. A less extensive hydrolysis might be beneficial for obtaining SPH with desired functional characteristics such as water binding, emulsification, and foaming [33,45,46]. SPH containing larger peptides might also be less bitter, considering that bitterness is mainly associated with low molecular weight peptides and hydrophobicity [6,11]. Sensory properties will in turn be decisive for consumer acceptance. Thus, size-based separation of peptides in SPH by UF has the potential to generate fractions with improved functional and sensory properties (R4 and R150) as well as fractions with increased bioactivity (P4).

2.4. Antioxidative Activity

The results from analysis of antioxidative activity in SPH, P4, R4 and R150 generated from enzymatic hydrolysis of saithe head (H) and backbone (B), and subsequent UF, is presented in Table 2. Three different in vitro assays were used to evaluate the antioxidative activity of SPH and UF fractions: ABTS, FRAP, and ORAC. These assays measure the ability of bioactive peptides to neutralize ROS by electron transfer, reduce Fe^{3+}, and neutralize ROS by hydrogen transfer, respectively. A combination of assays is preferred, as each assay measures a specific type of antioxidative activity within the assay-specific conditions and not the total antioxidative activity [24].

Table 2. Antioxidative activity ($\bar{x} \pm$ SEM, n = 12) of saithe protein hydrolysates (SPH), permeates (P4), and retentates (R4, R150) after membrane filtration with a 150 kDa cut-off ceramic membrane and 4 kDa cut-off PESH membrane. SPH were obtained from enzymatic hydrolysis of saithe heads (H) and backbones (B). Results from ABTS are given as μmol/g propyl gallate equivalents, FRAP, and ORAC are given as μmol/g Trolox equivalents. Results of Tukey`s post hoc grouping are indicated (H: h1–3, B: b1–3) for the individual analysis.

Sample	ABTS (μmol/g)	FRAP (μmol/g)	ORAC (μmol/g)
Hydrolysate	-	-	-
H(SPH)	58.33 ± 0.64^{h3}	5.16 ± 0.40^{h1}	380.50 ± 36.12^{h1}
B(SPH)	63.57 ± 1.20^{b3}	12.57 ± 0.40^{b2}	557.22 ± 72.47^{b2}
Permeate	-	-	-
H(P4)	42.38 ± 0.64^{h1}	8.06 ± 0.35^{h2}	394.97 ± 12.50^{h1}
B(P4)	55.37 ± 1.15^{b1}	10.70 ± 0.44^{b1}	547.79 ± 42.45^{b2}
Retentate	-	-	-

H(R4)	45.45 ± 0.94[h2]	7.58 ± 0.35[h2]	
B(R4)	59.72 ± 0.80[b2]	12.55 ± 0.28[b2]	354.39 ± 14.48[b1]
H(R150)	42.23 ± 0.64[h1]	14.70 ± 0.55[h3]	
B(R150)	56.07 ± 0.66[b1,b2]	15.55 ± 0.56[b3]	331.12 ± 52.82[b1]

Spawning was not found to affect the antioxidative activity of SPH, P4, R4, and R150, and thus, results are presented as average values for heads (H) and backbones (B) from October 2019 and January 2020 to include results of ANOVA and Tukey`s post hoc test (Table 2). SPH and UF fractions from backbone were found to have a significantly higher antioxidant activity compared to head measured by all assays (ABTS ($T(93) = 9.23$, $p < 0.05$), FRAP ($T(94) = 6.19$, $p < 0.05$), and ORAC ($T(70) = 1.40$, $p < 0.05$). This indicates that more antioxidative peptides are released from backbone during enzymatic hydrolysis compared to heads, which is somewhat contradictory to the observed higher prevalence of amino acids with known antioxidative activity in SPH from head (Section 2.2). However, this confirms the assumption that amino acid composition cannot be used as the sole indicator of antioxidative activity.

ABTS antioxidative activity was found to be significantly different between SPH and UF fraction for both head ($F(3,43) = 111.75$, $p < 0.05$) and backbone ($F(3,44) = 14.93$, $p < 0.05$). A Tukey post hoc test revealed that SPH had a significantly higher ABTS antioxidative activity compared to all other fractions. Both retentates (R4 and R150) from backbone SPH had significantly higher ABTS antioxidative activity compared to the permeate (P4), while no significant difference was observed between retentate R150 and permeate from head SPH. When measured by FRAP assay, a significant differences was observed between SPH and the UF fractions for both head ($F(3,43) = 106.76$, $p < 0.05$) and backbone ($F(3,44) = 21.49$, $p < 0.05$). Retentate R150 had the significantly highest FRAP antioxidative activity in both head and backbone. Retentate R4 and the permeate were both significantly higher than SPH for head, while the permeate was found to have the significantly lowest activity measured by FRAP for backbone. Regarding ORAC antioxidative activity, significant differences between fractions were observed only for backbone ($F(3,44) = 5.88$, $p < 0.05$), where SPH and P4 were found to have a significantly higher ORAC antioxidative activity compared to R4 and R150. This suggests that small peptides are important for ORAC antioxidative activity, but that a mixture of small and larger peptides are equally effective as the concentrated permeate.

No correlation was found between the results obtained from ABTS, FRAP, and ORAC. SPH was found to have the highest antioxidative activity when measured by both ABTS and ORAC but among the lowest when measured by FRAP. FRAP and ABTS assay both evaluate the ability of an antioxidant to reduce an oxidant with a comparable redox potential, and therefore, these results might be more comparable than results from ORAC assay, which measures the ability of an antioxidant to neutralize peroxide radicals by hydrogen donation [14,24,47]. However, while ABTS and ORAC assays are carried out at neutral pH, the FRAP assay is conducted in acidic conditions, which could suppress the antioxidative activity of bioactive peptides due to protonation [24,48]. This could explain the lower antioxidative activity of SPH found in the FRAP assay compared to the ORAC and ABTS assay. However, the acidic condition of the FRAP assay could be relevant when considering the stability and bioavailability of antioxidative peptides in the human gastrointestinal system. An instability of bioactive peptides in acidic conditions could also explain why R150 showed the highest antioxidative activity in the FRAP assay, as this UF fraction consisted of larger peptides that are possibly more resistant to changes in pH.

Another trend that can be observed from Table 2 is that the antioxidative activity of P4 was found to be equal to or lower than SPH, R4, and R150 in all three assays. These findings contradict what was the expected outcome of SPH processing. UF was applied to SPH with the intention of concentrating low molecular weight peptides, which are associated with higher bioactivity. Under this assumption, the expected result for antioxidative activity for UF fractions would be P4 >R4 >R150. SPH, having a smaller concentration of low molecular weight peptides relative to 4, would also be expected to exhibit lower bioactivity. Several studies on the UF of hydrolysates from various raw materials have confirmed this theory, including that of Farvin et al. [15], who found that applying UF to commercial cod hydrolysate significantly increased antioxidative activity. However, both Girgih et al. [14] and Picot et al. [16] found the unfractionated hydrolysate to have the highest antioxidative activity after UF of cod frame digests and commercial hydrolysates from cod and pollock skin, respectively. These studies both involved lower MWCO and lower molecular weight peptides, which indicates that further separation might not be effective for increasing bioactivity. This was further confirmed in this study by unpublished data on preliminary experiments with UF using 2 kDa MWCO membranes on SPH.

Regardless of the similar trends observed for SPH and UF fractions in this study, the measured antioxidative activity varies considerably among the assays. This inconsistency in the degree of antioxidative activity was also reported by Kristinova et al. [49] when comparing the results of Folin–Ciocalteu, DPPH, and ABTS assays, and it can be a result of limitations of the methodology of the assays as discussed above. Therefore, it can be challenging to compare results from various assays both within and among studies [22,24]. Furthermore, it is important to take into consideration that in vitro methods for assessing antioxidative activity cannot be directly extrapolated to functionality in food or the human body, as they do not consider intermolecular interaction, stability, or bioavailability [20].

The use of UF processing in an industrial setting would require an investment in both expensive equipment and expertise. Based on published results, it is also questionable whether such processing is likely to yield a product with increased bioactivity that could target a high-value market, which would be necessary to cover the extra cost. However, further studies are needed to refine and adapt the UF process to fish protein hydrolysates. In the event of an adapted process, where the fractions generated can be utilized based on their specific functional properties, e.g., water binding, emulsification, foaming, etc. for retentates and bioactivity for permeates, it would be possible to optimize the utilization of fish RRM proteins.

3. Materials and Methods

3.1. Chemicals

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate, ferric chloride, 2,3,5-triphenyltetrazolium chloride (TPTZ), 2,2′-azobis-(isobuttersa ureamidin)-dihydroclorid) (AAPH), propyl gallate, Trolox, acetate, phosphate, hydrochloric acid, methanol, and ethanol (Merck Life Science AS, Oslo, Norway) were used for the experiments and chemical analysis. All chemicals were of analytical grade.

3.2. Enzymatic Hydrolysis

The experimental procedure was conducted as described by Hjellnes, Rustad, and Falch [3] following the factorial design illustrated in Figure 7. Saithe were caught in Trondheimsfjorden, Norway, at two separate occasions: pre-spawning saithe in October 2019 (O) and spawning saithe in January 2020 (J). The fish was hand filleted, and the RRM was separated into three fractions: viscera (V), head (H), and backbone (B). Heads and backbones were used for further analysis.

The enzymatic hydrolysis was conducted on 500 g of minced RRM mixed 1:1 with preheated water (50°) for 60 min at 50 °C, physiological pH (pH = 6.0), and with the addition of 0.1% (1:1) papain (from Carica papaya, 1.5–10 U/mg, EC 3.4.22.2, Merck Life Science AS, Oslo, Norway) and bromelain (from Ananas comosus, 3 U/mg, EC 3.4.22.32, , Merck Life Science AS, Oslo, Norway) in bioreactors (Syrris Atlas, Model No. 2101000, Nerliens Meszansky AS, Oslo, Norway) with thermostats (Huber Ministat 125, Nerliens Meszansky AS, Oslo, Norway). A total of four RRM combinations (HO, BO, HJ, BJ) were hydrolyzed in two parallels, as presented in Figure 7. The enzymatic reaction was terminated by heat inactivation (>90 °C, 10 min), and the reaction mixture was transferred to 18 centrifugal tubes (50 mL), centrifuged (10,900 × g, 10 min, 20 °C) and frozen (–20 °C). The resulting three fractions, oil, hydrolysate, and sludge, were separated using a scalpel on frozen sample. Saithe protein hydrolysates (SPH) were further freeze dried (Labconco FreeZone 12, Labcono Corporation, Kansas City, MO, USA, −50 °C, <13.3 Pa) and frozen at −40 °C until further processing.

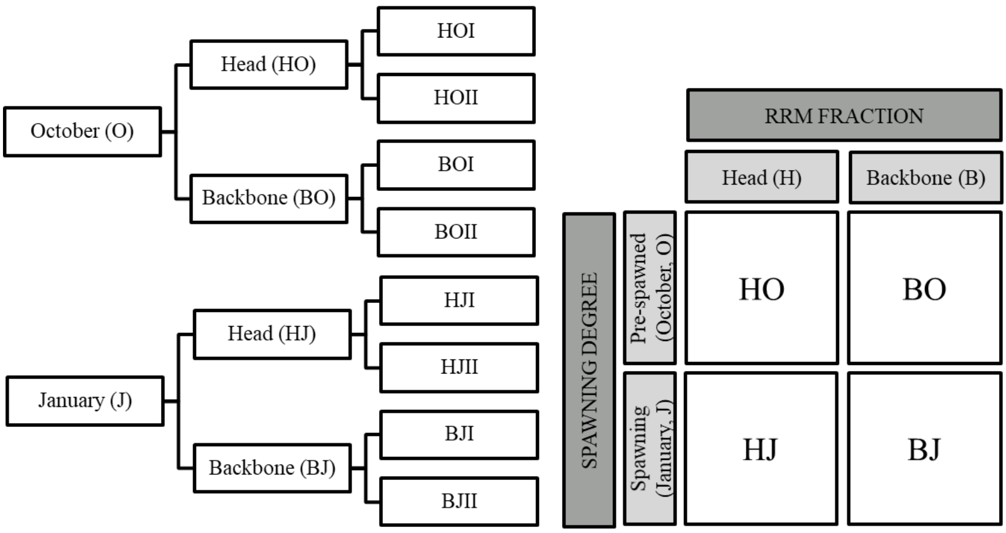

Figure 7. Schematic overview of the samples generated from (left), and the factorial design used for (right) enzymatic hydrolysis of saithe head and backbones from October 2019 (pre-spawned) and January 2020 (spawning) [3].

3.3. Membrane Ultrafiltration

Membrane ultrafiltration (UF) was conducted using a pilot-scale system (MMS Membrane Solution Triple System, DUE MILJØ AS, Halden, Norway) measuring 700 × 510 × 510 mm. The system had an 800 mL feed tank and a hold-up volume of 50 mL. A single membrane cell and three serially coupled membranes cells, each with an individual area of 28 cm^2, were used for the experiment. A ceramic membrane with a molecular weight cut-off (MWCO) of 150 kDa (Nadir®, MMS Nordic, Silkeborg, Denmark) was used for the single membrane cell, and PESH membranes (Nadir® UH004 *p*, MMS Nordic, Silkeborg, Denmark), with a thickness of 210–250 µm and a MWCO of 4 kDa, were used for the three serial coupled membranes.

SPH were resuspended in distilled water to a concentration of 1% (10 g/L) and then filtered through the ceramic membrane under a pressure of 500 kPa (Flux: 50 L/m^2h) at 21°C until hold-up volume was reached. The filtration yielded two fractions: a retentate with peptides larger than 150 kDa (R150) and a permeate with peptides smaller than 150 kDa (P150). P150 was subsequently filtrated through the PESH membranes (500 kPa, 21 °C), which generated two new fractions: a retentate with peptides larger than 4 kDa (R4) and a permeate with peptides smaller than 4 kDa (P4). R150, R4, and P4 were freeze dried (Labcono FreeZone 12, −50 °C, <13.3 Pa) and frozen at −40°C until further analysis. An overview of the processing steps and generated products is presented in Figure 8.

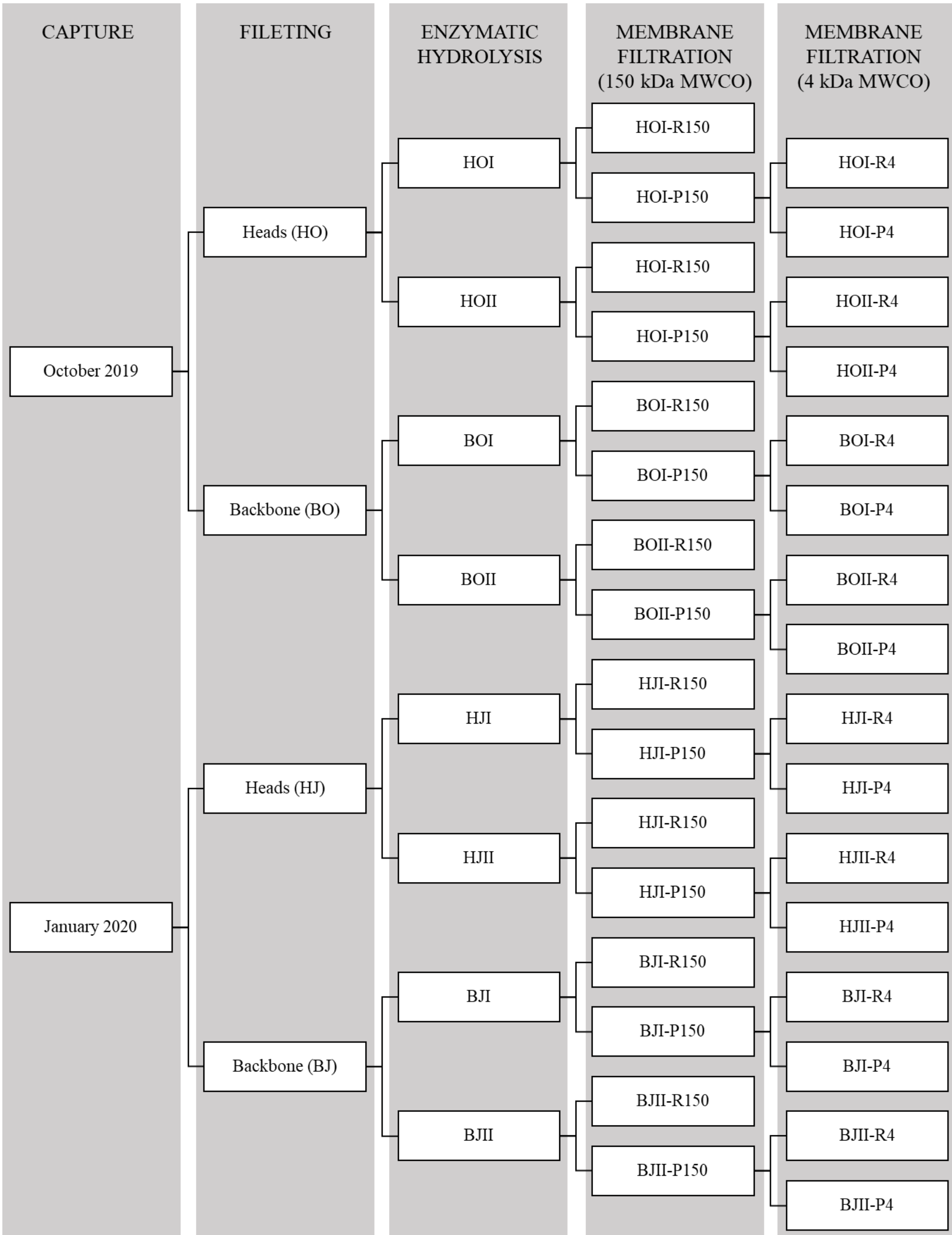

Figure 8. Overview of the processing steps and products generated from capture, filleting, and enzymatic hydrolysis of saithe rest raw material [3] and two membrane filtrations of saithe protein hydrolysates.

3.4. Dry Matter

Dry matter was analyzed gravimetrically according to AOAC [50]. The samples were analyzed in triplicates.

3.5. Antioxidative Assays

The antioxidative activity of saithe protein hydrolysates was analyzed by 2.2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activity, ferric-reducing antioxidant power (FRAP) assay, and oxygen radical absorbance capacity (ORAC) assay. ABTS was analyzed according to Re et al. [51] and Nenadis et al. [52] using propyl gallate as the reference compound [53]. FRAP was analyzed according to Benzie and Strain [54], and ORAC was analyzed according to Dávalos et al. [47] with previously described modifications [55] using Trolox as the reference compound. Values were reported as μmol/g propyl gallate equivalents and μmol/g Trolox equivalents, respectively. The samples were analyzed in triplicate.

3.6. Amino Acid Composition

Total amino acid composition was analyzed according to Blackburn [56]. Samples equivalent to 50 mg protein were hydrolyzed with 1 mL 6 M HCl for 22 h at 105 °C. The samples were neutralized (pH = 6.5–7.5) with NaOH and filtered through Whatman GF/C filters (25 cm, GE Healthcare, Boston, MA, USA) using a vacuum pump (Heto MASTER JET, Heto Lab Equipment AS, Allerød, Denmark). Following dilution, the samples were filtered through 0.2 μM syringe filters (VWR International, Oslo, Norway). HPLC (Ultimate 300 dionex, Nova-pak c184 μM 3.9 × 150 mm column, Thermo Fischer Scientific, Waltham, MA, USA) using a fluorescence detector (Rf 200) was performed by Siri Stavrum, NTNU. The samples were analyzed in triplicate.

3.7. Protein Efficiency Ratio (PER) Value

PER values were calculated as described by Šližytė et al. [37] using the following Equation (1):

$$PER = 0.08084[\Sigma AA_7] - 0.1094 \quad (1)$$

where ΣAA_7 = Thr + Val + Met + Ile + Leu + Phe + Lys.

3.8. Molecular Weight Distribution

The molecular weight distribution of SPH and P4 was analyzed by High-Performance Liquid Chromatography (HPLC) by Innolipid AS. Samples were dissolved in distilled water (10 mg/mL) and filtered through a low-protein-binding microfilter (0.22 μm) to remove large peptides and proteins. The analysis was performed on a Superdex column using a wavelength of 214 nm and hydrolyzed albumin as standard. The samples were analyzed in duplicate.

3.9. Statistical Analysis

All statistical analysis was conducted in SPSS software (IBM SPSS Statistics 27, 2020, International Business Machines (IBM), Armonk, NY, USA). Analysis of variance (ANOVA) and T-test were used for the comparison of means for >3 and <3 parallels respectively, assuming normal distribution and equal variance. Tukey’s post hoc test was used in combination with ANOVA to identify significant differences. Significance level was set to $p < 0.05$. Results are reported as mean values ($\bar{x}$) of [n] number of parallels ± standard error of the mean (SEM).

4. Conclusions

This study aimed at investigating the functionality of ultrafiltration (UF) as an industrial processing method, and its effect on the bioactivity of saithe protein hydrolysates (SPH). An initial filtration was performed on SPH with a 150 kDa MWCO membrane, creating a retentate (R150, >150kDa) and a permeate (P150, <150 kDa). UF was subsequently performed on P150 using a 4 kDa MWCO membranes, creating a new retentate (R4, >4 kDa) and permeate (P4, <4 kDa).

UF effectively concentrated small peptides in P4. UF processing was expected to increase the antioxidative activity of this peptide fraction, as small peptides have been associated with bioactivity. However, unfiltered SPH was found to have a similar or even higher antioxidative activity compared to P4 measured by ABTS, FRAP, and ORAC assays. These results indicate that concentrating small peptides by UF does not effectively increase bioactivity, and that bioactivity is dependent on several properties, including interaction with larger peptides. No correlation was observed between the results of ABTS, FRAP, and ORAC, and the measured antioxidative activity varied considerably among the assays. This indicates that it can be difficult to compare results among different assays both within and between studies.

The main product of enzymatic hydrolysis is SPH, while P4 can be considered the main product of UF when the goal is to concentrate small peptides. However, during both processing methods, the majority of the protein content ends up in fractions other than the main products. This highlights the importance of finding areas of application for the sludge fraction from enzymatic hydrolysis and the retentates from UF in order to make food production more sustainable by improving the utilization of saithe rest raw material.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical restrictions.

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