Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biotechnology and Food Science

Kristin Brustad

Lipid quality and utilization of rest raw material from fish processing

Based on the study of two cases: Norwegian herring and Indian surimi

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Supervisor: Eva Falch, Turid Rustad and Kari Helgetun Langfoss
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Abstract

The world's population is growing steadily which implies a world in need of more food. At the same time tons of rest raw material is being generated from the marine industry, and by optimal utilization these resources create value rather than waste. In Norway, most of the rest raw material is already being utilized to produce fish oil, fish meal or ensilage for the feed industry. As the rest raw material contains valuable lipids and proteins, it would be more profitable to process with the intent for human consumption. In India, large amounts of rest raw material are generated, although with a lower rate of utilization. The rest raw material presents a huge potential to reduce waste and produce valuable products.

The aim of this thesis was to investigate lipid quality in rest raw material from two cases, Norwegian herring and Indian surimi production, and it was investigated if enzymatic hydrolysis and membrane filtration could contribute to a better utilization of these resources. The thesis has been part of the ReFood project, to strengthen the global collaboration between Norway and India. Most of the work was conducted at The Central Food Technological Research Institute (CFTRI) in Mysore, India.

A combination of fieldwork and laboratory work was used to approach the thesis. Three different rest raw materials were investigated, crude oil and fish protein hydrolysates (FPH) from herring and from Indian surimi FPH was produced. Analyses of total lipid content, fatty acid profile, and lipid classes was performed for herring, whilst for surimi a predefined "ideal process" of enzymatic hydrolysis based on scientific literature was brought from Norway to perform in India. Based on our fieldwork and a questionnaire sent to different Indian actors a SWOT-analysis was conducted to enhance the knowledge of the potential of Indian rest raw material utilization.

The results showed a lipid content of 13.8 % (dry weight) in the FPH from Pelagia which was higher than anticipated. The oil had a high content of polyunsaturated fatty acids and would therefore be susceptible for lipid oxidation. The omega-3 fatty acid content was high in both crude oil (6.33 % EPA and 6.81 % DHA of total FA) and FPH (~7.5 % of total FA for both EPA and DHA) and is within expected values of herring based on seasonal variations. From membrane filtration fraction B (retentate 150 kDa) had the highest lipid concentration (20.54 %) and indicates that the phospholipids are retained in this fraction and was highly susceptible for lipid oxidation. Rest raw material from surimi had a general low fat content. As for the "ideal process" of enzymatic hydrolysis several challenges were uncovered. The most challenging steps were maintaining of cold chain, issues in the separation of oil from hydrolysates and an overall different laboratory practice. The rest raw material was challenging to work with (skin, bones, heads etc.) in addition to being highly perishable, which emphasizes the importance of a maintained cold chain. The results from the SWOT-analysis showed that there is a big potential for utilization of rest raw material in India. Through my exchange to India I have experienced, observed and concluded that there are different challenges one encounters when utilizing this type of raw material in India compared to Norway. New insight has been gained that would not have been as accessible if I had not traveled and experienced through fieldwork and the practical laboratory work. Future work on the utilization of rest raw materials from surimi production can use this work as a basis for moving forward towards the goal of increased utilization of marine rest raw material.

Sammendrag

Verdens befolkning er i stadig vekst som innebærer et økende behov for mer mat. Årlig skapes det tonnevis av marint restråstoff, og ved en optimal utnyttelse av disse ressursene vil det skapes verdi fremfor avfall. I Norge utnyttes allerede det meste av restråstoffet til produksjon av fiskeolje, fiskemel eller ensilasje til fôrindustrien. Restråstoff inneholder verdifulle lipider og proteiner, og det vil være mer lønnsomt å prosessere for menneskelig konsum. I India skapes det store mengder restråstoff, men med lavere utnyttelsesgrad. Restråstoffet har et stort potensial for å redusere avfall og produsere verdifulle produkter.

Hensikten med denne masteroppgaven har vært å undersøke lipidkvaliteten i restråstoff fra to ulike caser, norsk vår-gytende sild og fra indisk surimiproduksjon. Det ble også undersøkt om enzymatisk hydrolyse og membranfiltrering kunne bidra til en bedre utnyttelse av disse ressursene. Masteroppgaven har vært en del av prosjektet ReFood for å øke det globale samarbeidet mellom Norge og India. Arbeidet er i hovedsak utført ved forskningsinstituttet CFTRI i Mysore, India.

En kombinasjon av feltarbeid og laboratoriearbeid ble brukt til å løse oppgaven. Tre forskjellige restråstoff ble undersøkt, råolje og fiskeproteinhydrolysat (FPH) fra sild, og fra indisk surimiproduksjon ble FPH produsert. Totalt lipidinnhold, fettsyreprofil og lipidklasser ble analysert for sild, mens for surimi ble en forhåndsdefinert "ideell prosess" for enzymatisk hydrolyse basert på vitenskapelige artikler tatt med til India for å forsøke å utføre ved CFTRI. Basert på vårt feltarbeid i forbindelse med utveksling og et spørreskjema sendt til forskjellige indiske aktører ble det utført en SWOT-analyse for å øke kunnskapen rundt potensialet for utnyttelse av indisk marint restråstoff.

Resultatene viste et lipidinnhold på 13,8% (tørrvekt) i FPH fra Pelagia og var høyere enn forventet. Oljen hadde et høyt innhold av flerumettede fettsyrer og dermed utsatt for lipidoksidasjon. Omega-3-fettsyreinnholdet var høyt i både råoljen (6,33% EPA og 6,81% DHA av totale fettsyrer) og FPH (~ 7,5% av totale fettsyrer for både EPA og DHA), og ligger innenfor forventede verdier for sild basert på sesongbaserte variasjoner. Fettinnholdet i restråstoff fra surimi var generelt lavt, men bar preg av lite homogenitet og mye variasjon i prøvematerialet. Vedrørende den "ideelle prosessen" for enzymatisk hydrolyse ble flere utfordringer avdekket. De mest utfordrende punktene var opprettholdelse av kuldekjede, problemer ved separering av olje fra hydrolysater og generelt en annen laboratoriepraksis. Restråstoffet var vanskelig å jobbe med ettersom det besto mye av skinn, bein og hoder. I tillegg er det et lett-bedervelig råstoff som understreker viktigheten av at kuldekjeden holdes intakt. Gjennom dette prosjektet og utvekslingen til India har jeg opplevd, observert og konkludert med at det er forskjellige utfordringer ved å bruke denne typen råstoff i India sammenlignet med Norge. Ny innsikt er innhentet som ikke ville vært like tilgjengelig dersom jeg ikke hadde reist til India og fått erfaringer gjennom feltarbeid og det praktiske laboratoriearbeidet. Fremtidig arbeid innen utnyttelse av restråstoff fra surimiproduksjon kan bruke dette arbeidet som grunnlag for å kunne nå målet om økt utnyttelse av marint restråstoff.

Preface

This master thesis is part of the two-year M.Sc. program Food and Technology at the Norwegian University of Science and Technology (NTNU), Department of Biotechnology and Food Science. The thesis has been part of the Indian-Norwegian cooperation in the two projects ReFood and ReValue initiated by SINTEF and NTNU. As part of the project an 11-week exchange to India took place, and most of the laboratory work has been conducted at CFTRI in Mysore, India. CFTRI is one of the top research facilities within food technology in India.

I am grateful for the opportunity to partake in the ReFood and ReValue projects, and all the possibilities it has given me this year. The exchange to CFTRI has been an educational, interesting, testing and memorable experience. To experience the differences between Norway and India not only in culture, but in science and research has been a fun, intriguing and, at times, a frustrating experience.

First and foremost, I would like to thank my supervisors Eva Falch and Turid Rustad, who have been of great value for me in both academia and guiding me in how to best solve this thesis. I would also like to thank Kari Helgetun Langfoss and PhD candidate Veronica Hammer Hjellnes for their help and expertise. From CFTRI I would like to thank Dr. N.M Sachindra and Dr. Ajay W. Tumaney for their guidance and support during my exchange.

Writing this master has been challenging at times, as the exchange to India brought more challenges than first anticipated. Unforeseen things occurred, communication and HSE were not without complications, and as a result the thesis has continuously altered throughout the thesis. Although challenging, and frustrating at times, it has been a good experience that I have learned a lot from.

Lastly, I would like to thank my friends and family who has been supporting me throughout my studies.

Thank you!

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Abbreviations

ALA	α-linolenic acid
CFTRI	Central Food Technological Research Institute
CFS	Central Instruments Facility and Services
CSIR	Council of Scientific and Industrial Research
DAG	Diacylglyceride
%DH	Degree of hydrolysis
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
EFSA	European Food Safety Authority
FA	Fatty acid
FAME	Fatty acid methyl esters
FFA	Free fatty acids
FPH	Fish protein hydrolysate
GC	Gas chromatography
GC-FID	Gas chromatography-flame ionization detector
GDP	Gross domestic product
HSE	Health safety and environment
ICMR	Indian Council of Medical Research
IIT	Indian Institute of Technology
LC-PUFA	Long-chained polyunsaturated fatty acids
MAG	Monoacylglyceride
MF	Microfiltration
MUFA	Monounsaturated atty acids
NMR	Nuclear magnetic resonance spectroscopy
NTNU	Norges teknisk-naturvitenskapelige universitet
PUFA	Polyunsaturated Fatty Acids
SD	Standard deviation
SDG	Sustainable development goals
SFA	Saturated fatty acids
SPE	Solid phase extraction
SWOT	Strength, weaknesses, opportunities and threats
TAG	Triacylglyceride
TCA	Trichloroacetic acid
TLC	Thin layer chromatography
TNBS	Trinitro-benzene-sulfonic acid
UF	Ultrafiltration

1. Introduction

The world's population is growing steadily which implies a world in need of more food. The fisheries of the world are highly exploited resources, with most species at the peak of their regenerating capacity. Overfishing is a threat and as fish resources are not unlimited, it is of great importance to utilize the resources of the catch in a more optimal manner. This can be done by utilizing the rest raw material generated when processing the fish. Rest raw material, or by-products, can be defined as everything remaining of the fish once the usual commercial products such as round, fillet, beheaded or gutted fish are accounted for, and then after further processing can be sold. (Rustad et al., 2011) The majority of the rest raw material of herring is processed for the animal and fish feed industry as it is a good source of protein and crude oil and is utilized either by producing fish meal and oil, or ensilage (Carvajal et al., 2015, RUBIN, 2012). However, by utilizing these resources with the intent for human consumption rather than animal feed the profit would increase. The resources could be implemented as human food, nutraceuticals, functional food, and cosmetics.

In 2018 Norway exported 292 090 tons of herring with a value of 2.63 billion NOK, and herring accounted for 34,8 % of the total income from the pelagic sector. Approximately 54 % of all the herring exported was whole, unprocessed fish, while the rest is processed in Norway before being exported or sold in Norway. (Norges Sjømatråd, 2019) The processing is mainly filleting which generates a significant amount of rest raw material, and in 2009 a total of 291 000 tons of rest raw material was produced from the pelagic sector alone. Most of this is already being utilized as previously mentioned, however, seen as the rest raw material from filleting is of food-grade quality it indicates that high quality products can be produced from it. It is desirable to maintain the high quality into new products produced from the rest raw material. In the salmon industry products have shown to be of high quality when produced from rest raw material taken immediately after gutting, and thus several factories have adjoining locations for both slaughtering and rest raw material utilization. When maintaining the high quality, desirable fish protein and fish oil can be produced. Relatively new technology allows for controlled hydrolysis and fish protein hydrolysates (FPH) can be produced. These protein hydrolysates can have nutritional, functional and bioactive properties that makes them valuable and interesting in a variety of markets. (Carvajal et al., 2015, Slizyte et al., 2010)

Health claims is in many ways a tool to educate people into taking more informed food choices.(Jones and Jew, 2016) The Codex Alimentarius defines health claims as any representation that suggests, states or implies that there is a connection between a food or a food constituent and health (Codex Alimentarius, 1997). False health claims can mislead consumers, and due to this one cannot promote health claims that are not scientifically based. There are countless studies on the health effects of omega-3 fatty acids (FA) which especially shows a correlation between eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and maintaining good brain, heart and eye health. The European Food Safety Authority (EFSA) has set guidelines for a daily intake of EPA and DHA needed to achieve positive health effects. For a normal, healthy person this is set to 250 mg. An example of an EFSA approved health claim is that omega-3 FA EPA and DHA

help to maintain normal heart, brain and vision function with a daily intake of 250 mg. (Jones and Jew, 2016, VNP AS, n.d)

According to the Norwegian dietary advice from 2011 it is suggested that one should consume around 300-450 grams fish each week. This is equivalent to 2-3 servings of fish each week. These recommendations regard both lean and fatty species, although it is recommended that at least 200 grams from the suggested intake should come from fatty species. In Norway roughly half of the population consume less then what is recommended (Helsedirektoratet, 2011). Fish in general is a well-known source of omega 3 FA, and omega-3 has shown to have numerous of health benefits, including positive impacts on hypertension, obesity, diabetes, inflammatory disease, eye disease, neurological disease and especially cardiovascular disease. As omega-3 also is found in plants (ALA), it is important to highlight that it is the marine, long-chained polyunsaturated FAs (LC-PUFA) found in fatty species that in general are associated with cardiovascular health improvements (Yashodhara et al., 2009, Jones and Jew, 2016).

India has the second biggest population on earth and is situated by the Indian Ocean with a coastline that contribute to a large marine landing. The marine fishing industry is important as it provides food to millions of people, creating employment and exportation along the coastline. In 2017 the estimated marine landing in India was at 3.83 million tons, an increase of 5.6 % from 2016 (Fishery Resources Assessment Division, 2018). Today India is one of the world's leading economies and is Asia's third largest after China and Japan. Since the millennium, the country has had one of the fastest growing economies in the world, mainly due to development in production technology, IT sector and an increase in foreign investments. Nevertheless, due to its enormous population (1.3 billion) it is one of the poorest countries in the world when dividing the gross domestic product (GDP) on all inhabitants. The economic growth has not been distributed evenly, and there is great economic disparity among the people of India (FN-Sambandet, 2019b). As India is a country in growth, they present a huge potential and opportunity to make a difference regarding better utilization of marine rest raw material. In Norway most of the rest raw material is already being utilized, and the challenge lies in how the value can be increased.

The earth consists of 70 % ocean and 30 % land, yet 95 % of all food produced comes from land-based production and only 5 % comes from the ocean. Around 2 % of human calorie intake and 15 % percent protein intake comes from the ocean. In order to feed the estimated 9.1 billion people that will be roaming the earth by 2050 the food production has to be increased tremendously and it is important to look towards the oceans. However, new contributions from marine food production should come from lower in the food chain than what is practiced today, and already existing production needs to find new solutions to increase sustainability. As previously mentioned, a way of fully utilizing resources is by transforming products generally regarded as waste into profitable, valuable products preferably for human consumption. (Torrissen et al., 2018)

1.1. Aim of thesis

The aim of this thesis was to investigate lipid quality in crude oil and fish protein hydrolysates obtained from rest raw material of Norwegian spring-spawning herring and from lipid containing fractions of Indian surimi production. Exploring if enzymatic hydrolysis and membrane filtration can contribute to a better utilization of rest raw material regarding lipids and proteins in the two chosen cases has also been investigated. Two completely different raw materials were used, and it also aims to look at the differences regarding utilization of rest raw material in Norway and India. This thesis has been a part of the international projects ReFood and ReValue to strengthen the relationship between Norway and India. As the project evolved it became apparent that an exploratory approach was necessary, and different directions had to be taken consecutively based on discoveries from early processes and analyses. As a result, the thesis underwent continuous alterations and became more practical and experiential in terms of looking at the challenges and opportunities in utilization of marine rest raw material in India.

1.1.1. Objectives

Main objective

Examine if it is possible to better utilize the different side streams of herring and Indian surimi with focus on lipid quality in both crude oil and in hydrolysates, and if enzymatic hydrolysis and membrane filtration can contribute to this.

Intermediate objectives

- Determine lipid composition of crude oil from herring and determine the lipid content of the FPH and investigate if it is at an acceptable level (below 0.5 % in the FPH).
- Observe how phospholipids remaining in the FPH behave during membrane filtration
- Highlight challenges in utilization of rest raw material and the general mentality regarding these resources in our chosen case in India. Investigate where the challenges and opportunities lie.
- Uncover challenges in trying to meet a predefined "ideal process" of enzymatic hydrolysis based on scientifically published procedures and standards used in Norway when attempting to follow this process in India.

2. Background and motivation

2.1. ReFood and ReValue

ReFood, together with ReValue, are two projects initiated by SINTEF Ocean and is a cooperation between Norway and India that aims to strengthen the global bio-economy and improve resource utilization by using innovative technology. The aim is to develop an international partnership between India and Norway. The projects are funded by The Research Council of Norway as part of their INTPART program (SINTEF, 2018). This master thesis is part of the Re-Food project, which mainly focuses on building a long-term collaboration between the Norwegian and Indian partners. The Norwegian partners are SINTEF Ocean and NTNU, whilst the Indian partners are IIT, Amity University, BITS Pilani and CSIR-CFTRI. Other industrial and government actors in both countries are also represented (SINTEF, 2017).

Through this project, part of the master was conducted at CFTRI (Central Food Technological Research Institute) located in Mysore, India, together with fellow master students Frida Holm Larsen and Sara Aakre. The same raw material has been the basis of all three masters although with different focus areas, namely lipids, proteins and antioxidative properties. Throughout the thesis it will be referred to their work. In preparation for this exchange we participated in a workshop in Mumbai where communication with Indian industry, professors and students was established. The workshop and newfound network proved valuable for the upcoming work, along with other company visits and gatherings in both Norway and India. All different workshops and company visits are further described in chapter 3.

Regarding raw material, Pelagia Sildoljefabrikk in Bodø provided both crude oil and FPH from herring, and the Indian surimi factory Kaiko has provided different segments of rest raw material of pink perch used in their production. A complete description is found in section 3.1.2 and 3.2.1.

2.2. Rest raw material in a circular bio-economy approach

Bio-economy can be defined as an economy where the fundamental building blocks for energy, chemicals and materials are obtained from renewable biological resources, such as animal and plant sources. A bio-economy supports sustainability from both an environmental and economic perspective, if implemented in an intelligent manner. Utilization of side-streams from fish processing, such as rest raw material, is a bioeconomic solution where nothing goes to waste (McCormick and Kautto, 2013).

Marine rest raw material constitutes an important value-creating resource in the Norwegian aquaculture and fisheries industry. Most of it are already being exploited in a good manner, but there is great potential to increase the utilization rate and to produce more value-added products. The Norwegian aquaculture industry have in general had an increased focus on rest raw material utilization, and a growing marine ingredient industry is eager to increase the amount of rest raw material used in their production. Marine oils and proteins from both the pelagic and whitefish sector are included in a circular economy as an important feed ingredient for farmed salmon. (Richardsen et al., 2017)

2.2.1. Sustainable development goals

In 2015 the United Nations made a list of 17 sustainable development goals (SDG) that function as the world's common work plan in order to get rid of poverty, combat inequality and to stop climate change by the time we reach 2030. There are especially two SDG that are relevant for this thesis, number 12: Responsible production and consumption, and number 14: Life below water. Another SDG that is relevant is number 2: Zero hunger. With the increasing world population it is highly important to fully utilize the resources that already are in circulation (FN-Sambandet, 2019a). In the SDG progress report from Lipinski et al. (2017) SDG target 12.3 is discussed in terms of food loss and what has been done to reach the goal. SDG target 12.3 aims to halve the global food loss per capita at both retail and consumer level. Reduction in food losses from production, including post-harvest food losses, and supply chains are also part of the target. The report is divided into three segments, where they emphasize the importance of setting targets, measuring food losses and lastly act. Without action there will be no change. Naturally, exactly what needs to be done varies from country to country. In developing regions, such as India, most of the food loss occurs at the production and storage level. A key to reducing this food loss is investment in the infrastructure to improve processing, storage and transportation. When looking at greenhouse gas emissions on a worldwide basis India is the third-largest emitter. However, if global food loss and waste were a country it would surpass India. (Lipinski et al., 2017)

2.2.2. Norwegian spring spawning herring (*Clupea harengus L.*)

Norwegian spring spawning herring (herring) is a pelagic fish with a general high fat content, although with significant seasonal variations. It spawns in February-March along the Norwegian coastline and is the stock that forms the foundation of Norwegian small herring. The most rapidly growing of these is mature after 2-3 years. However, they can live for 25 years and get a length of approximately 40 cm. They always move in shoals and are quite easy to spot when they are moving in the water. The species are planktonic, and their diet consist of crustaceans, roe and fry. Herring has been of significant economic value for Norway since the 13th century, when together with Denmark, supplied Europe with herring. It has often been referred to as the "silver of the sea" which not only refers to its appearance but also its economic importance. Herring is a good source to vitamin A, D and B12, and rich in omega-3 FA. Although, as previously stated, with great seasonal variations regarding the lipid content. (Grimsmo, 2011, Norges Sildesalgslag, 2019).

2.2.3. Indian Surimi

As part of the ReValue project it has been of relevance to do research on surimi production. Seeing how the production can be improved by implementing better technology and maintaining the cold chain, but also raw material utilization has been of interest.

Surimi is a fish paste rich in proteins and can be defined as refined myofibrillar proteins from fish produced through several processes. The different processes involved include beheading, gutting, filleting, removal of bones, washing, removal of water, refining and mixing with cryoprotectants before freezing. The most important step is washing, to ensure an odor- and colorless surimi. By efficient washing many of the issues regarding odor, color and taste are minimalized or eliminated completely. The myofibrillar proteins make up approximately two-thirds of the minced meat, whereas the last one-third

consists of blood, fat, myoglobin and sarcoplasmic proteins that impair the final quality of the surimi. Thus, washing increases the quality as the undesired constituents are removed while the myofibrillar proteins are concentrated and the frozen shelf life in turn is extended. The washing process includes several washing cycles, although how many cycles and the amount of water used varies with type of species, freshness, equipment and desired final quality. Usually, the ratio of water to raw material varies between 5:1 and 10:1 and consequently big amounts of wastewater are produced. Surimi production uses a variety of white fish species, and surimi is used as a base in a variety of products, where the perhaps most well-known usage is in crabsticks. Surimi is also used as shellfish substituents or in traditional Japanese cuisine. The production of surimi originated in Japan as early as in the 11th century and has since expanded throughout the world. In Asia, countries such as Thailand, Vietnam, China, Indonesia and India are big producers of surimi (Park et al., 2013, Park and Morrissey, 2000) Indian surimi production is rather novel, and did not start until the 1990s. Since then the production has grown, and in 2011 the total surimi production across India was at 58 000 ton, of which 53 000 ton was exported to a variety of countries. (Park et al., 2013)
In India surimi is produced from a variety of Indian species. A few of the more common species are pink perch (*Nemipterus japonicus)*, Barracuda (*Sphyraena sp.)* and croaker fish (*Johnius sp*.). (Naik, 2019)

2.2.3.1. Pink perch *(Nemipterus japonicus)*

N. japonicus, also known as pink perch or rani fish amongst Indians, is a marine fish typically found in the Pacific and Indian Ocean. Pink perch is a huge bycatch in the shrimp industry in India and widely used in surimi production. It contributes with approximately 3.5 % of the total marine landing in India. Pink perch is common in the tropical Indo-west pacific region, and has become a valued food fish in different parts of the world. However, prior to 1993 the fish was undesired and considered rubbish by many. After 1993 when surimi production started up in India, pink perch became important to the industry because of its low cost, white meat and abundance of it. Pink perch is a lean species and not necessarily a good source to omega-3 (Naqash and Nazeer, 2013, Singh and Balange, 2005).

2.3. Lipids

Lipids is the common term for fats and is made up of nonpolar substances (triacylglycerides, diacylglycerides, monoglycerides and sterols) but also more polar substances such as phospholipids and free fatty acids. Lipids also include other subgroups such as carotenoids, waxes and steroids. These are all naturally occurring molecules that are soluble in organic solvents, and insoluble in water. All organisms consist of proteins, carbohydrates and lipids as they are an essential organic chemical in the biomass, and energy storage is the main biological function of lipids. Lipids are also important in cell membranes, as they function as structural components. (Ficken, 2014, SNL, 2018, Manirakiza et al., 2001)

2.3.1. Fatty acids

Fatty acids (FA) are a group of organic substances and are the main constituents of fat and fat-like substances. All FA consist of a chain of carbon atoms, where at one end there is a carboxylic acid group (COOH) and a methyl group (CH_3) at the other end. FA are divided in saturated and unsaturated. In saturated FA (SFA) each carbon atom will

bind two hydrogen atoms, except the carbon at the end. In unsaturated FA there will be one or more carbon atoms with only one hydrogen atom attached to them. These carbon atoms have a double bond which separates saturated from unsaturated FA and the unsaturated can be divided into monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) depending on how many double bonds they contain. Unsaturated FA have different chemical and physical properties than saturated FA, such as a lower melting point and is more susceptible to lipid oxidation. Fats that contain much unsaturated FA are liquid at room temperature and is known as oils whereas fats with a high content of SFA will be solid. (SML, 2018, Pedersen et al., 2013)

2.3.1.1. Omega-3

Omega-3 are unsaturated FA with desirable health benefits. There are three types of omega-3 that are most relevant regarding human physiology: α-linolenic acid (18:3, ALA), eicosapentaenoic acid (20:5, EPA) and docosahexaenoic acid (22:6, DHA). ALA is mainly found in vegetable oils and nuts and is the shortest FA of the three, whilst EPA and DHA are long-chained polyunsaturated FA (LC-PUFA) and is originally of marine origin such as fish, marine mammals and microalgae (figure 1). Out of the three, EPA and DHA are of greatest importance regarding health benefits. Although, these are not essential FA and do not have to be introduced through the diet, seen as ALA can be transformed to EPA and DHA in the body. However, this process is highly ineffective that it is recommended to eat foods rich in EPA and DHA. (Khan et al., 2015, Havforskningsinstituttet, 2015)

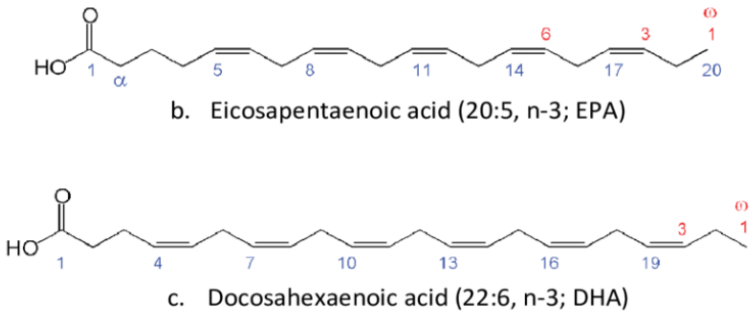

Figure 1: Chemical structure of EPA and DHA (Khan et al., 2015)

In the typical Indian diet, the main sources of fats are vegetable oils, dairy products and ghee (clarified butter). In 1990 several dietary advices were published, aiming to reduce the intake of ghee and other traditional SFA oils such as groundnut- and coconut oils and replace them with rich PUFA oils such as sunflower oil. Omega-3 LC-PUFAs are of marine origin, and except from specific coastal regions, marine ingredients are not typical in the Indian diet. The Indian Council of Medical Research (ICMR) has through dietary recommendations aimed to increase the intake of omega-3 PUFAs in Indian diets, although without any distinct solution on how to achieve this. A big majority of Indians follow a vegetarian diet and do not consume omega-3 LC-PUFAs, and amongst non-vegetarians the general marine intake is regarded as negligible. It has been suggested

that the daily intake of PUFAs for Indians are 20-50 mg a day at most, which is far below the recommended dietary allowances of 200 mg a day (Mani and Kurpad, 2016). The advice to increase omega-3 PUFAs in the diet did not specify to increase the intake of LC-PUFAs, although it could be of interest to investigate.

2.3.2. Lipid classes

Lipids can be divided into classes where triacylglycerols (TAG) and phospholipids are two of the more common classes. TAGs consist of a glycerol and 3 FA. TAG is a neutral lipid class, and close to all commercially oils and fat from plant and animal origin of importance consist almost exclusively of TAG. The 3 FA most often differ from each other and there are different variations of TAG, and the lipids in fish are unique as they contain much of the omega-3 LC-PUFAs. Lipases are lipid-cleaving enzymes that catalyzes hydrolysis of ester bonds in TAGs that results in degradation products such as diacylglycerides (DAG), monoglycerides (MAG), and free fatty acids (FFA) can be formed. DAGs consists of a glycerol and two FA and is formed during degradation of TAG as one FFA is split off. (Christie, 2003, Lande et al., 2018, Hauge, 2018, Ditlefsen, 2016)

Phospholipids are more polar lipids than TAGs and consists of two FA, one phosphate group, glycerol unit and one polar molecule. Phospholipids are an important component of cell membranes. Phospholipids (figure 2) are amphiphilic which means they have polar heads and non-polar tails. The polar heads are hydrophilic and will associate with water because of dipole-dipole interactions, whilst the non-polar tails are hydrophobic and will turn away from water molecules. In aqueous environment phospholipids will spontaneously gather, with the polar heads facing the water and nonpolar FA facing inwards and forms what is called micelles. (Bailey, 2018, Pedersen et al., 2013, Zahrabi et al., 2016)

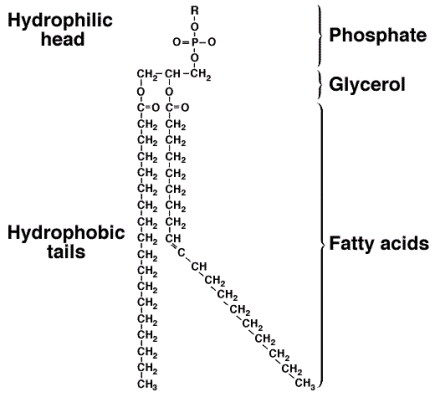

Figure 2: Molecular structure of phospholipids (Zahrabi et al., 2016)

2.3.3. Lipid quality

Lipid oxidation is an important factor in the deterioration of food and comes second to microbial activity on spoilage in any food. Oxidation gives the undesired sensory properties associated with rancidity, and may contribute to destruction of vitamins, discoloration, loss in nutrient value and limited shelf-life. Lipid oxidation mainly involves the reaction of oxygen with unsaturated FA and is a degradation process that occurs at the double-bonds in glyceride molecules. The more double-bonds and unsaturated the FA is, the more susceptible for oxidative degradation they become. Omega-3 as LC-PUFAs are highly susceptible for oxidation along with FFA. The content of FFA in foods affects both taste and shelf-life and can be measured as a sign of quality. (Schaich et al., 2012, Sherwin, 1978, Hauge, 2014). Lipid oxidation can be measured through e.g. peroxidation value (PV) and anisidine value (AV) that measures the primary and secondary oxidation products of the oil. By controlling certain important factors such as exposure to oxygen and temperature, the risk of oxidation can be reduced. Temperature should be kept as low as possible during processing and transportation, and it is shown that material exposed to high temperature during processing might experience that the oxidation rate is increased and result in low stability and quality. (Carvajal et al., 2015, Miller, n.d)

2.3.4. Lipids in herring

Herring has a rather high lipid content, however there are significant seasonal variation in the lipid content and composition. The lipid content might vary from 5 % in spring and ≤20 % around august and contains around 18 % in the wintertime (October-January) Herring accumulates the fat mostly in the light muscle but also underneath the skin. Herring caught in the fall/wintertime will contain 50-70% of the total lipid content in these tissues. In addition herring will have fat deposits in head, abdominal epithelium, dark muscle and backbones (Grimsmo, 2011).

2.3.5. Lipids in Indian fish species

A study conducted by Rai et al. (2012) investigated lipid content in three different Indian marine species. Lipids from head, meat and waste was analyzed from *N. japonicus*, Indian mackerel *(Rastrelligar kanagurta)* and Indian oil sardine *(Sardinella loniceps).* The total lipid content in head, meat and waste samples ranged from 4.3-13.6 %, 2.53-10.97 % and 2.7-15.1 %, respectively.

2.4. Fish protein hydrolysates

Processing of rest raw material from fish can result in high-quality fish meal, fish oil and ensilage when handled properly. These products are commonly used in the feed industry, although more profitable products could be produced. A more profitable way of utilizing these proteins are through fish protein hydrolysis. Hydrolysates are proteins that have been broken down into peptides of varying size, either chemically or enzymatically, and hydrolysis is traditionally performed to either retrieve proteins from rest raw material of to discover functional properties of underutilized proteins. FPH are food ingredients that have become increasingly more important as due to their unique properties, high nutritional value and health beneficial effects (Pires and Batista, 2013, Kristinsson and Rasco, 2000, Mazorra-Manzano et al., 2018).

2.4.1. Enzymatic hydrolysis

Enzymatic hydrolysis can be used to modify or improve the functional, physiochemical and sensory properties of the proteins without damaging the nutritive value. Often, the protein absorption will also be enhanced. Adding enzymes will give good control of the hydrolysis, and thus the properties of the hydrolysates (Kristinsson and Rasco, 2000). In order to run metabolic reactions, fish and all living organisms requires enzymes to function as catalysts. These are endogenous enzymes, and only certain enzymes are active when they are needed. When death occurs the control of the endogenous enzymes which automatically will begin to deteriorate the fish, also known as autolysis. (Mukundan et al., 1986)

More than 3000 different enzymes exist, and different enzymes works on different substances. The substance of which an enzyme functions is called the substrate of the enzyme, although an enzyme can have several substrates. Enzymes are often highly specialized, both in terms of which reactions they catalyze and what substrates they accept. The area of which the substrate attaches to the enzyme is called the active site, and when the substrate attaches to the enzyme an enzyme-substrate complex is formed. The substrate is then subjected to energy changes and is cleaved into a new product. Then it detaches, and the enzyme finds a new substrate to attach and the process continues. The reaction rate depends on the concentration of enzymes and substrates, and usually the enzyme concentration is decisive of the reaction rate, which is a consequence of the substrate molecules being in a large excess compared to enzyme concentration. (Kierulf et al., 2018, Whitehurst and van Oort, 2009, Bhagavan and Ha, 2015)

Proteases are enzymes that initiate proteolysis by hydrolysis. Alcalase, Flavourzyme, Neutrase and Protamex are common commercially produced proteases used for fish protein hydrolysis. Each of the proteases has optimal conditions for hydrolysis that should be followed when performing enzymatic hydrolysis. For Alcalase, the optimal conditions are pH of 8.0-9.5 and temperature between 50-60 °C (Aluko, 2018).

Pelagia Sildoljefabrikk produces FPH from rest raw material of herring, and in figure 3 a simplified flowchart of their production is shown. Freshly caught herring is fileted and rest raw material consisting of heads, scales, bones, trimmings, blood and viscera is delivered to Pelagia. It has previously been found that production of herring filet generates between 36 % to 54 % rest raw material from whole weight. There are great seasonal variations at play, and trimmings and viscera constitute most of the rest raw material generated (Falch et al., 2006). The delivered fractions are mixed together without addition of water. The mixture is then heated to 85 °C and once it reaches this temperature a tri-canter separates crude oil, sludge and stick water. The separated crude oil constitutes 70 % of the oil in the material. The stick water and sludge go through a flash cooling before going into hydrolysis reactors and enzymatic hydrolysis is performed. Enzymes are added, and once the hydrolysis is finished it is heated to 85 °C to inactivate the enzymes before separation. The enzymes used in the process is unknown as requested by the producer.

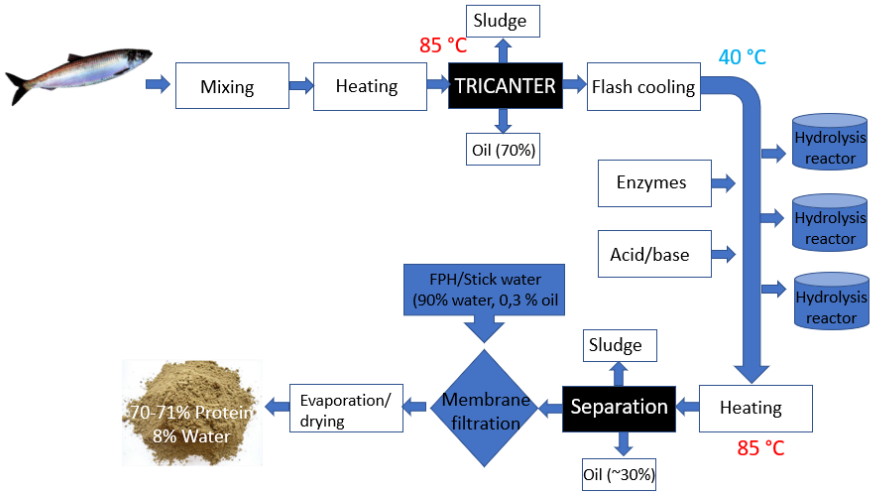

Figure 3: A simplified flow chart of Pelagias fish protein hydrolysate production based on factory visit in November 2018. Membrane filtration step is not currently performed at the factory but might be performed in the future. (Hjellnes, 2019)

2.4.2. Membrane filtration

Membrane filtration is a modern physiochemical separation technique based on differences in permeability as a separation mechanism and provides an alternative to centrifugation. During filtration the content of the feed stream is pumped against the membrane. As shown in figure 4, the membrane is semipermeable which means that some components in the feed stream easily permeates through the membrane, while it will be less permeable, or even impermeable, to other components. Thus, during running permeable components passes through the membrane (permeate) while impermeable components will remain on the other side of the membrane (retentate). Consequently, the permeate will be relatively free of impermeable components, while the retentate has concentrated the content of impermeable components (Crittenden et al., 2012).

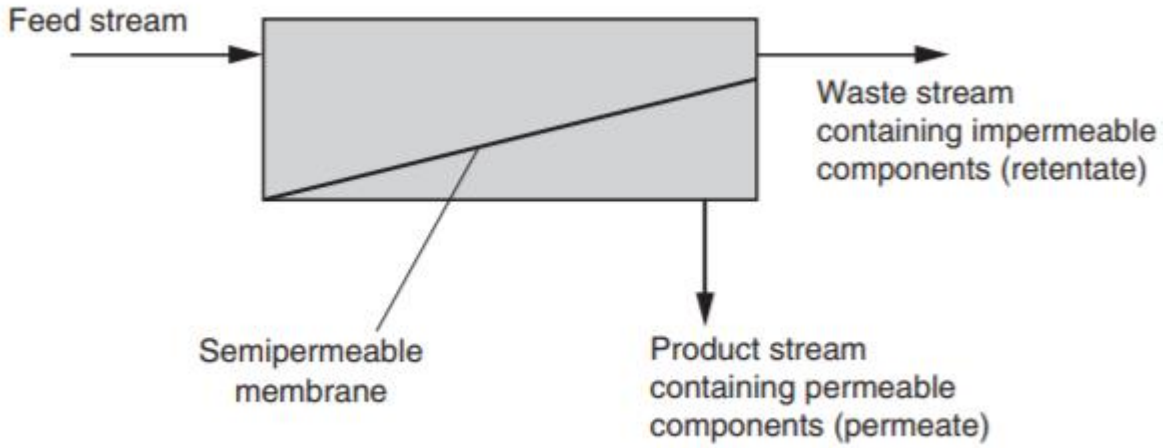

Figure 4: Flow chart of membrane filtration through a semipermeable membrane (Crittenden et al., 2012)

Membrane filtration uses microfiltration (MF) and ultrafiltration (UF) membranes. An important parameter in membrane filtration is the size of the material that is being retained by the membrane. This is called retention rating and differs between MF and UF. In MF this is designated by the pore size in µm and for UF it is the molecular weight cut-off (MWCO) in Daltons, although it is common to use pore sizes for UF as well. The pore sizes of MF and UF differ between 0.1 µm and 0.01 µm, respectively. Occasionally, a membrane experiences fouling which indicates loss of performance. This can occur when dissolved substances attaches on the external surfaces of the membrane, within the pores or blocking the pores of the membrane. Fouling is possible the number one issue regarding membrane filtration as it impedes operation, but also affects the cost-effectiveness as it is difficult to remove by washing and the membrane's lifetime becomes reduced. A parameter that can reduce the risk of fouling is the transmembrane pressure, which is the difference in pressure between the feed and permeate borders of the membrane. If the pressure is below 1 bar the risk is reduced, and for most membrane filtration systems the transmembrane pressure lies between 0.2-1 bar. Another way of reducing the risk of fouling is by using cross-flow filtration, which is a filtration technique where the feed stream is pumped parallel to the membrane surface at a high velocity to prevent build-up of retained substances at the surface of the membrane (Crittenden et al., 2012, Cheryan, 1998).

2.5. Fieldwork and qualitative analytical methods

Fieldwork, or field research, consist of qualitative methods to collect information by observing, interacting and understanding people in their natural environment. A few methods are direct and participant observation, qualitative interviews and case studies. Direct observation is gathering information through observation, without the researcher interfering. In participant observation the researcher can participate in discussions and get a deeper understanding of its subjects and is more involved in the process. Qualitative interviews are normally close-ended questions, although they can be either informal, standardized, semi-structured or open-ended or a combination of all. Qualitative interviews are a way of providing big amounts of data for the researcher to sort through. Case studies are in-depth analysis of either a situation or an event. (Bhat, n.d)

For most qualitative research exploratory design is of relevance. An exploratory design means that choices are made during the project, based on new insight that is gained. Problem statements may have to be readjusted as the project progresses. This can happen as the researcher during the study becomes aware of which aspect of the original problem that will provide relevant knowledge. From a scientific point of view this might be perceived as choices that threatens the validity of the research. Although, seen from a philosophy of science point of view this sort of flexibility is an advantage, as qualitative methods can open for new and unexpected knowledge. If all choices were made in advance, this would not be possible. (Skolbekken et al., 2010)

Information gathered from these qualitative methods can ultimately be placed into a SWOT-analysis to get a better understanding of the different opportunities and threats of either a country, company or organization. SWOT is an acronym for strength, weakness, opportunities and threats. A SWOT-analysis is used to investigate and comprehend a company's strong and weak sides, and what can be their market opportunities and threats. The method normally consists of one external and internal analysis, where the

external analysis looks at opportunities and threats regarding economic, technological, political, customer and supplier aspect etc. for the company. The internal analysis looks at strength and weaknesses within the company where the goal is to identify conditions that can be developed into lasting competitive advantages and also conditions that may weaken their competitiveness. (Vikøren, 2018)

3. Material and methods

In this thesis two cases have been studied to investigate whether the utilization of rest raw material can be improved regarding lipid quality. The two different cases investigated are:

1. Production of herring in Norway and rest raw material from this processing, looking at herring crude oil and FPH
2. Rest raw material from Indian surimi production and challenges with the utilization of this material.

Acquisition of knowledge

Two different approaches have been applied in order to solve this thesis statement. As displayed in figure 5, laboratory work and fieldwork together with qualitative analytical methods taken place in both Norway and India have been used to outline the challenges of a thought "ideal process" and as the basis of a SWOT-analysis.

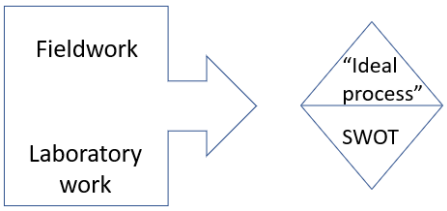

Figure 5: Approaches to obtain results. Fieldwork and laboratory work together form the basis of a "ideal process" and its challenges and a SWOT-analysis

Fieldwork

In order to obtain valuable knowledge and insight into the Indian perspective of rest raw material utilization several measures in terms of company visits and excursions were conducted. In addition to the Indian perspective, it was important to learn how the situation is in Norway. All the different measures along with what the intended goal from that specific measure was is presented in table 1.

Table 1: Approach to obtaining insight to help achieve main objective

Measures to gain insight	*Goal*
Workshop Mumbai	- Establish communication with Indian partners - Insight into rest raw material utilization in India - Indian industry
Visit to Kaiko surimi factory	- Insight into Indian surimi production. - Tour of the process plant, from washing to finished product in freezer storage.
Visit to Pelagia Bodø Sildoljefabrikk	- Insight into Pelagias production and general FPH production in Norway.
Business summit New Delhi	- Attend seminar on sustainable seafood production - Insight to Indian and Norwegian industry and how they get benefit from a cooperation - The prime minister of Norway, Erna Solberg had an official visit to India where the summit was on her agenda. - Visit to Amity university
Student exchange to CFTRI	- Exchange to Indian research facility January-March 2019. - Take a good procedure from raw material to finished product and an "ideal process" of how its normally performed in Norway – bring said procedure to India and investigate if it can be done under different circumstances. - Establish contact with Indian university
Questionnaire regarding rest raw material utilization in India	- First-hand knowledge from Indian industry and universities. Obtain information from students, professors and industry regarding rest raw material handling in India. - Exploratory design. Bringing qualitative analyses into analytical procedures to highlight different aspects of the findings.
SINTEF meetings and knowledge bank	- As ReFood is initiated by SINTEF they have been providing valuable information and guidance. - Several analyses performed by SINTEF upon returning from India

Workshop Mumbai and visit to Kaiko surimi factory

On November 28th-29th 2018 the second symposium for the ReFood and ReValue projects was arranged in Mumbai, India. During the short course of this symposium substantial information was gathered regarding both projects and what is being done in Norway and

India, but also a quick glimpse into what living in India would be like. As part of the symposium we were invited to a surimi factory, Kaiko, located north-east of Mumbai. We were given a tour of the production plant and had a meeting with the daily manager, Asif Naik. Later, it was arranged that they will provide us with rest raw material from their production throughout the exchange in India. The rest raw material would be sent to the institute (CFTRI) were all the analysis where to be conducted.

Visit to Pelagia Bodø

On November 5th, 2018 a visit to Pelagia Bodø Sildoljefabrikk took place. I travelled together with PhD candidate Veronica Hammer Hjellnes, and we had a meeting with daily manager Jon Vestengen, and got a tour of the factory. The trip was sponsored by Pelagia and gave a good overview of the production line, and a picture of how large this industry is. We arranged that Pelagia would send us weekly samples up until the middle of December to have enough material to work with for all three master students.

Business summit New Delhi

The prime minister of Norway, Erna Solberg, had an official visit to India in the beginning of January 2019. On January 7th The Indian-Norwegian Business Summit for Sustainable Growth 2019 was held, where we got to partake in the segment on sustainable seafood. Many interesting presentations was held, and we had a reencounter with Daily manager Asif Naik from Kaiko whom we previously had met in Mumbai. After the summit the opening ceremony of the new Norwegian embassy in New Delhi took place. We received invitations to participate both in the summit and the inauguration of the new embassy. It was an incredible opportunity and experience that we are truly grateful to have been able to participate in.

CFTRI

As part of the fieldwork an 11-week exchange to India was conducted. The exchange took place at CFTRI in Mysore, Karnataka in southern India. CFTRI is part of CSIR (Council for Scientific and Industrial Research) which have institutions all over India. At CFTRI Dr. N.M Sachindra, head of Meat and Marine Sciences Department, was our supervisor. The department has a high focus on rest raw material. As I am investigating the lipids, I was conducting most of the laboratory work at the Department of Lipid Science under Dr. Ajay W. Tumaney. The production of FPH took place at Dr. Sachindras department, and I was shifting back and forth between laboratories.

Questionnaire regarding rest raw material utilization in India

Qualitative analytical methods were implemented in this thesis to help highlight challenging aspect when doing research abroad. A questionnaire was prepared to provide answers with an Indian perspective along with our own findings from our observational work. As it reflects on both fieldwork and results from laboratory work it was natural to describe it last and is found in section 3.3

Laboratory work

This section is divided by the two cases studied in this thesis. All laboratory work performed on herring both in India and Norway are presented first, followed by all laboratory work performed on surimi. A simple description of what was performed in India is shown in figure 6, which also shows that both crude oil and FPH was investigated for herring, while for surimi only FPH was produced.

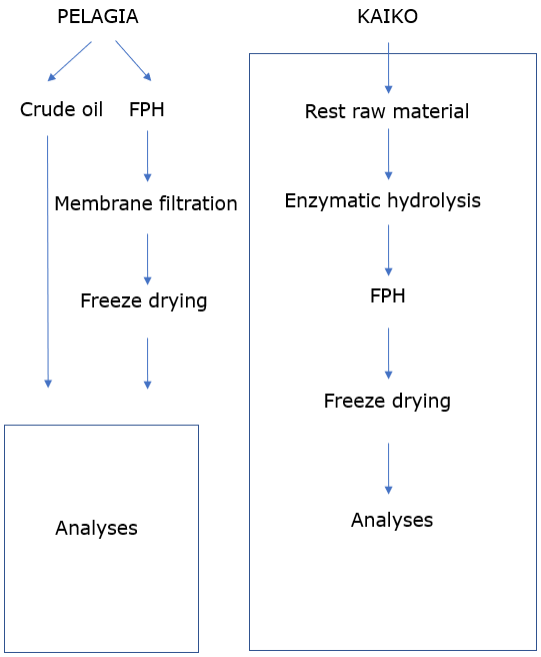

Figure 6: A simplified flow chart describing what was done in India in the two different cases. The boxes represent what was performed in India. It gives an idea of the differences between the cases and visualize how they are not comparable.

3.1. Herring

3.1.1. Overview of the work conducted

The work started in Norway with hydrolysates received from Pelagia. The hydrolysates were fractionated through membrane filtration and freeze dried for conserving before analysis, which is described in section 3.1.2. The samples were brought to India where different analyses was carried out. In figure 7 a brief overview of the planned analyses is presented. All analyses on crude oil are listed, along with fat extraction of FPH before the same analyses was run on the different fractions obtained from membrane filtration.

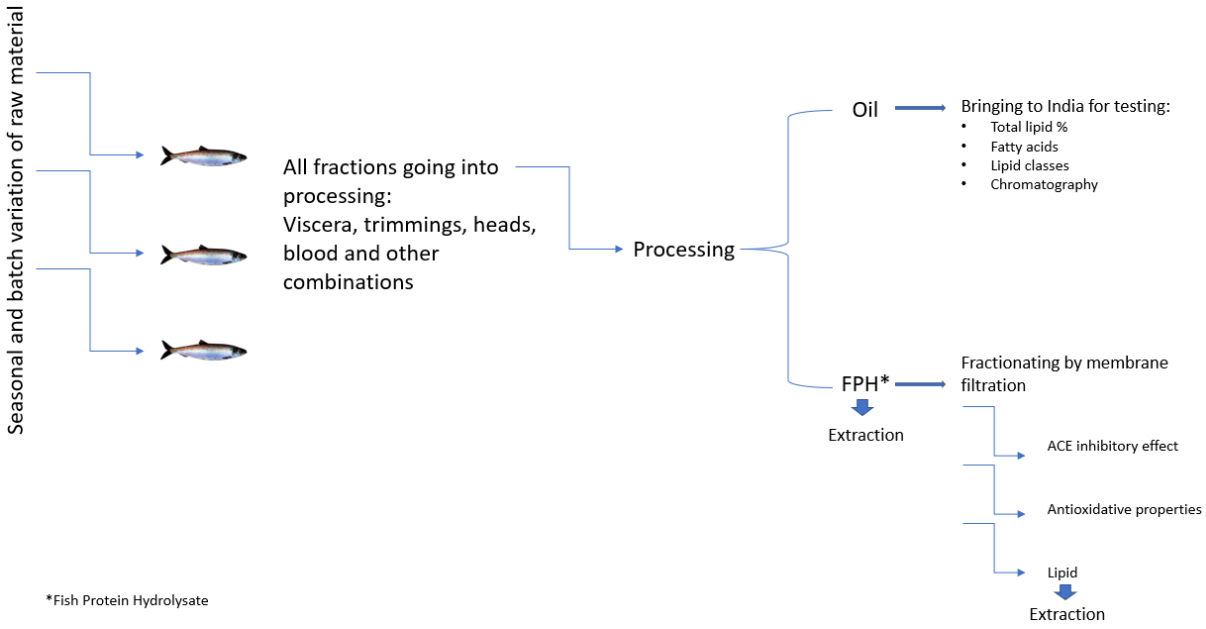

Figure 7: A brief overview of the processing of rest raw material of herring, and where in the process the different analysis has been conducted.

3.1.2. Raw material from Pelagia

Pelagia Bodø Sildoljefabrikk contributed with both hydrolysates and crude oil from herring rest raw material. Due to seasonal variations in the fat content of herring it was preferably to receive a weekly batch from November to December. This was unfortunately not possible, and only one batch was received. As this material was to be the basis of three master theses, it meant that there was some scarcity and one had to take measures to make sure material was not wasted. This also meant that there was not enough raw material to run duplicates on some analysis which have led to uncertainties in the performed analyses.

Crude oil was received together with FPH on the 21st of November 2018 and was produced on the 19th. The batch received consisted of 0.5 L crude oil and 1.5 L FPH, and the samples were sent and kept in frozen storage to obtain initial quality. The crude oil

was kept in frozen storage until departure for India, but due to the long journey and pitstop in New Delhi it was not kept under ideal storage conditions and will not be representative of Pelagia. The FPH was thawed and separated into three containers, whereas one was put directly in a freeze drier to perform total lipid and dry matter content while the remaining FPH was filtered through membrane filtration.

3.1.3. Fractionation of FPH by membrane filtration

Membrane filtration was used to fractionate the FPH before analyses was run. The filtration was performed at NTNUs food processing laboratories (Akrinn) on an MMS Triple System. Due to a high dry matter content in the hydrolysate, it had to be diluted before filtration. The dilatation rate was 2:1 (400 ml H_2O:200 ml FPH). This was well within the limit of 800 ml for the feeding tank of the MMS Triple System. The instructions for the MMS system were followed systematically.

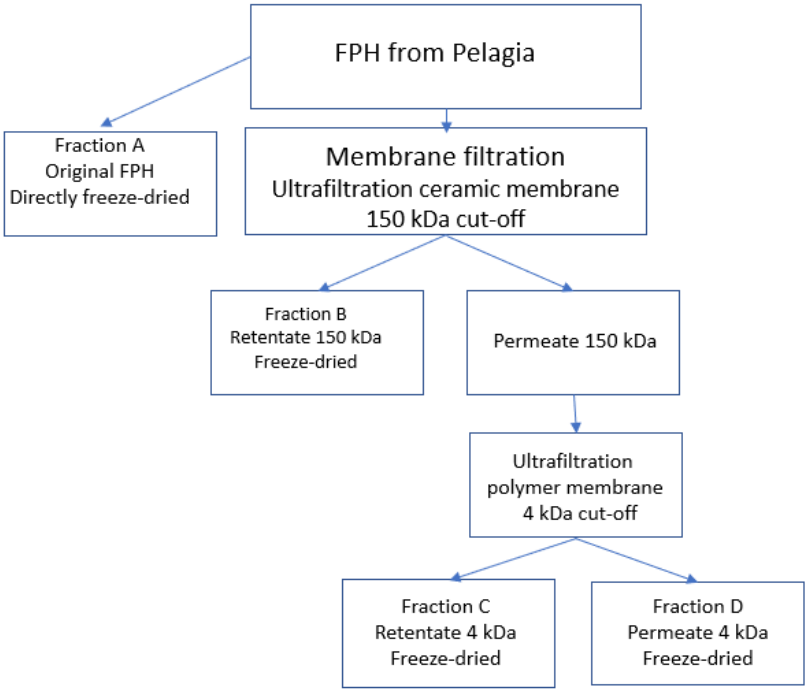

Figure 8: Flow chart of membrane filtration on FPH from Pelagia and the different fractions obtained in the process

Firstly, a ceramic membrane (TAMI Industries, 150 kDa, area 28 cm^2 x 1) was used. The ceramic membrane functions as a clarification step and is used to remove larger particulates. This process lasted an entire day, and the filtration eventually had to be stopped before it had finished. Thereafter the retentate and permeate were stored refrigerated overnight. The following day ultrafiltration was performed by using polymer flat sheet membranes (NADIR UH 004, Microdyn Nadir, 4 kDa cut-off, area 28 cm^2 x 3 = 84 cm^2) to filtrate the permeate obtained the previous day. This in turn gave a new retentate and permeate. In total four different fractions were obtained. The original hydrolysate, 150 kDa retentate, 4 kDa retentate and 4 kDa permeate which is clearly shown in figure 8. The entire process was repeated once to obtain enough samples to bring to India. As the feeding tank has a maximum capacity of 800 ml, the process had to be performed in two turns. All the samples were from the same batch and therefore

the corresponding fractions were mixed. All fractions where ultimately freeze dried using a FreeZone 12l Cascade Console Freeze Dry System. At first the samples would not sublimate and boiled instead. It was decided to keep the samples at -80 °C overnight and run the freeze drier again. Due to the low temperature it took longer for the samples to be dried, approximately 72 hours. After being freeze dried the samples were stored in a -20 °C freezer in ziplock plastic bags until departure for India. Naturally, these samples have a strong odor. To contain the smell, the samples were vacuum packed the day before departure. One batch of original FPH was freeze dried to analyze dry matter content and total lipid content without being fractionated. This took around 48 hours. Normally one can log the fluctuations and action of the MMS system, however, this did not work properly and none of the activities were logged successfully. Due to this the flux value is not known for the filtration.

Analyses on crude oil

3.1.4. Thin layer chromatography

To visualize the lipid classes in the crude oil thin layer chromatography (TLC) was performed. Chromatography is a collective name of a large group of separation methods that's based on the substances being separated continuously between a stationary and mobile phase. The mobile phase is used to transport the substances through the stationary phase, and the separation occurs due to the different chemical and physical properties of the substances. In TLC the stationary phase is applied as a thin layer on a plate, often silica, while the mobile phase is a liquid. After loading samples onto the plate, it is placed in a closed TLC chamber containing the mobile phase, or solvent system. The mobile phase will begin to run upwards together with the different substances that is transported with the mobile phase. Before it has run all the way up the plate is removed and dried. TLC is a rapid and often inexpensive tool that can be used for a rapid screening of the composition of a sample (Wibetoe, 2018).

Crude oil is highly concentrated and needs to be diluted. 10 µl crude oil was dissolved in 500 µl $CHCl_3$. The results showed that this concentration was too high, and the sample had to be diluted further until precise plates could be developed. Rice bran oil which contain TAG, DAG and FFA was used as standard (10 µl:500 µl $CHCl_3$). After all samples were diluted the TLC silica gel (Merck, TLC Silicagel 60 F_{254}) was prepared. A 20 cm*20 cm sheet was cut into four squares of 10 cm*10 cm. One cm from bottom was marked on both sides, and five 1 cm long lines were drawn along the markings. Each of the samples were to be placed on one of these lines. Thereafter the silica gel was put in an oven at 105 °C for 10 minutes to activate the plate. Simultaneously as the plate got activated, the solvent for the TLC chamber was made. The solvent system consisted of Petroleum Benzine (Merck, Mumbai), Diethyl Ether (SRL, $C_4H_{10}O$) and Glacial Acetic Acid (SRL, $C_2H_4O_2$), (35 ml:15 ml:0.5 ml), blended in a measuring cylinder. The solvent was slowly poured into a completely dry TLC chamber. The lid was closed and stayed shut for at least 15 minutes for the chamber to get saturated. The samples were loaded onto the activated TLC plate. For the standard, 7 µl was placed. For the samples, labelled H1 and H2, were placed with 5 µl and 10 µl, respectively. Once saturated, the TLC plate was placed inside the chamber using a pair of tweezers. After the solvents had run, the plate was removed, dried with a hair dryer, and placed in an iodine chamber to develop the plate. A quick snapshot was taken as the iodine is not permanent. To make it permanent it was placed in a charring solution and put in a heating oven at 105 °C for the plate to

dry. The charring solution was ready made and kept in the lab (0.63 g $MnCl_2$ mixed with 60 ml H2O and 60 ml MeOH, before addition of 4 ml sulfuric acid mix).

To quantify the TLC plates a standard curve of TAG was made (TAG: 30 µl Triolein to 1 ml $CHCl_3$. New dilution 16.6:483.4.). A TLC plate was run in an equal solvent system as the one previously used, with increasing TAG concentrations from 5 µl-25 µl loaded onto the plate. Using a gel doc (Bio-Rad) and the program ImageLab, TLC plates could be digitalized and quantified using the ImageJ software.

3.1.5. Determination of fatty acid composition

Determination of FA composition of crude oil was performed at SINTEF Ocean by engineer Merethe Selnes. Fatty acid methyl esters (FAMEs) was created before analyzing in a gas chromatograph with flame ionization detector (GC-FID).

Fish protein hydrolysates

3.1.6. Determination of total lipid content of FPH

To measure the total lipid content of the hydrolysates, fat was extracted using a modified version of the Bligh and Dyer method (Bligh and Dyer, 1959). The total lipid content of the original freeze dried FPH was determined at Gløshaugen using a macro method of Bligh & Dyer. Due to shortage of time and material this could not be performed for the remaining three fractions of hydrolysates, and thus a micro method was performed on the remaining fractions while in India.

In the macro method, the hydrolysates were analyzed in triplets. The samples (5 g) were weighed precisely in centrifugation tubes, and homogenized (Ultra-Turrax, 2 minutes) with water (16.0 ml), methanol (40.0 ml) and chloroform (20.0 ml). All homogenization was performed in fume hood with centrifuge tubes on ice to minimalize vaporization and increase accuracy. Chloroform (20.0 ml) was added and homogenized for 40 seconds, then water (20.0 ml) was added before homogenization for 30 seconds. The samples were then centrifuged for 15 minutes which gave three fractions. The lipids accumulate in the chloroform phase and 2 ml from this phase was pipetted into weighed test tubes. The chloroform was evaporated with nitrogen on a heating block, leaving only the lipids in the test tubes. The tubes where then weighed again to measure the lipid content. The remaining steps of the analysis was completed by laboratory engineer Oskar Speilberg, and the extracted oil was brought to India for further analyses.

In India the same method was applied for the remaining three fractions, though with slight alterations in the execution. The material obtained in Norway was to be used by all three master students, and as previously mentioned it was not of an adequate quantity, and one had to be sparse. In accordance with Dr. Tumaney it was decided to downscale to 1/10 of the already modified method. The amount of lipid required to run the planned analysis is very low, and it was estimated that there would be enough oil extracted to do further analyses. Samples of 0.5 g were accurately weighed into 50 ml Falcon tubes. Although not ideal, Falcon tubes were used as those were suited for the centrifuge and the only tubes that could contain the final volume of the samples. Ideally it should have been glass. The macro method is intended for samples in wet weight (w/w) and could naturally be downscaled due to a higher concentration in dried samples. The ratio of solvents in B&D should be 1:2:1 (H_20:MeOH:$CHCl_3$). 2 ml of H_20, 4 ml MeOH and 2 ml

$CHCl_3$ was added to the Falcon tube. The sample was homogenized (IKa Ultra-Thurrax, 5 speed, 2 minutes). Another 2 ml of $CHCl_3$ was added and homogenized (40 seconds) before 2 ml of H_20 was added and homogenized (30 seconds). Different phases in the sample was visible at this point. The sample was then centrifuged (Thermo Scientific, Sorvall ST 8R, 15 min, 3400 x g) which gave three phases. The lipids are dissolved in the bottom $CHCl_3$ layer and is transferred to an already weighed micro centrifuge tube (mct) using a pipette. The micro method gives very little yield, and therefore the entire $CHCl_3$ phase was pipetted into the mct and evaporated using nitrogen gas. Once all $CHCl_3$ had evaporated the mct was weighed for gravimetric calculation. Based on initial sample weight and total weight the percentage of lipid content was calculated.

The same procedure was followed for all three fractions, although fraction D had a stickier texture than the rest and for it to dissolve it had to be placed in an ultrasonic water bath (Branson 2800, 30 °C, 5 minutes) prior to homogenizing, seen as a vortex did not dissolve the sample completely.

3.1.7. Separation of lipid classes

The lipids extracted from the original FPH and from each fraction of membrane filtration was used. The extracted oil was separated into three lipid classes, neutral lipids, glycolipids and phospholipids using solid phase extraction (SPE) through a Sep-pak column. 2 ml of $CHCl_3$ was placed in a column (Supelclean™ LC-Si SPE Tube, SUPELCO) to equilibrate the column. Once the chloroform had flown through, the column was placed in a preheated oven at 65 °C for 30 minutes to get activated. In the meantime, samples and solvents was prepared. Four tubes labelled neutral lipids, glycolipids, phospholipids and unbound lipids were placed on a rack (figure 9). Each lipid class required a different solvent. 5 µg oil was diluted in 2 ml $CHCl_3$ and added to the activated column and placed above the tube labelled unbound. For neutral lipids 6 ml of $CHCl_3$ was added. For glycolipids 6 ml of acetone:isopropanol (9:1) was added. Phospholipids were extracted with 6 ml of MeOH. Once the oil was separated the samples were dried in a vacuum concentrator (Eppendorf Concentrator Plus) for approximately 1.5 hours. All samples were dried in the vacuum except phospholipids. The dried lipid content was transferred to vials using $CHCl_3$ and dried completely with nitrogen. The vials were weighed before and after for gravimetric calculations. All samples were stored at –21 °C before TLC was run.

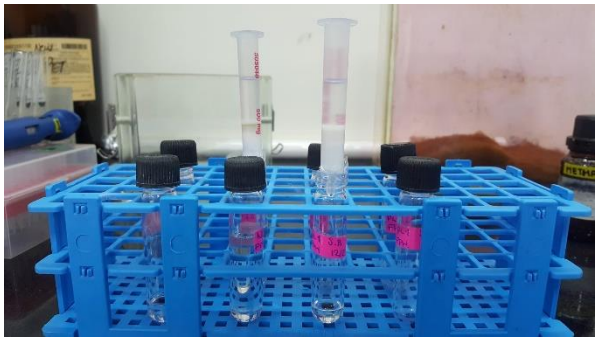

Figure 9: Separation of lipid classes by solid phase extraction

TLC on various lipid classes

Neutral lipids were run on a TLC using the same solvent system as described in section 3.1.4. Four samples were loaded onto the TLC plate. A TAG standard, the extracted oil, unbound lipids and neutral lipids. The solvent systems used were based on internal procedures. For glycolipids the solvent system was Acetone:Acetic Acid:H_2O (50:1:0.5). Phospholipids was run in a solvent system of $CHCl_3$:MeOH:Acetone:Glacial Acetic Acid:H_2O (25:5:10:7.5:2.5).

3.1.8. Determination of fatty acid composistion

Before performing gas chromatography (GC) it is essential that FAMEs are created. Preparation of FAMEs was done by following the method described by Sreedhar et al. (2017) which is based on the transesterification of FA with Boron trifluoride (BF_3) and methanol.

The samples of neutral lipids, glycolipids and phospholipids obtained from the solid-phase extraction was used. 200 µl neutral and glycolipids were blended with 50 µl internal standard (Heptadecanoic acid, C17:0), whilst for phospholipids 300 µl was blended with the same internal standard due to its lower concentration. Then 1 ml BF_3-methanol (13-15% BF_3 basis, Sigma, USA) was added and vortexed for 3 seconds. The samples were kept in a water bath at 60 °C for 30 minutes, and immediately afterwards kept on ice for a minimum of five minutes for the vapor to condensate. After the samples had cooled 1 ml H_20 and 1 ml hexane was added and vortexed for 3 seconds. The samples will now have two phases, an aqueous phase and an upper hexane layer where the lipids have dissolved. Using a small pipette, the upper hexane layer was collected and transferred to GC-vials. To ensure no water was present, a pinch of sodium sulphate was added to remove moisture, before transferring to a new GC-vial. This was only done for the samples where water was clearly still present in the vial. All samples were completely dried with nitrogen gas, sealed with parafilm and kept in frozen storage until GC was run. GC-FID was run on Shimadzu Gas Chromatograph GC-2014, using a temperature program showed in table 2. When placing each sample 0.1 µl was injected using a syringe.

Table 2: Temperature program for Shimadzu Gas Chromatograph GC-2014. Start temperature at 120 °C. Increasing 8 degrees a minute until it reaches 175 °C. Then increasing 3 degrees a minute until reaching final temperature at 220 °C where it holds for 2 minutes.

Rate (°C/min)	*Temperature (°C)*	*Hold time (min)*
-	120	0
8	175	0
3	220	2

In order to quantify the different FA, the internal standard (C17:0) was used. By using the formula below the amount of each FA was calculated.

$$\mu g\ FA = \frac{area\ of\ unknown\ FA\ x\ 50\ \mu g\ \text{internal standard}}{area\ of\ internal\ standard}$$

3.2. Surimi

3.2.1. Characterization of raw material

On January 23rd rest raw material from surimi factory Kaiko was delivered at CFTRI. The material was brought from Mumbai to Bangalore by flight, and from Bangalore to CFTRI by truck. All the different fractions were frozen, and an enzymatic hydrolysis was performed to produce FPH before further analysis was conducted on both proteins and lipids. The material received was of pink perch and was received in three different fractions: head and viscera (HV), skin and bones (SB), and refined waste (RW). Indian species in general have a low-fat content, and the fat will mostly be found in the viscera of these species. At Kaiko a decapitating machine is used, and head and viscera are removed in one step. To characterize the chemical composition of the raw material, four different analysis was conducted as shown below. Internal laboratory procedures were followed for all four analyses.

Moisture content

Moisture content was calculated gravimetrically. 10 g raw material was accurately weighed in already weighed glass petri dishes and labelled properly. The samples were placed in a heating oven at 105 °C overnight. The samples were collected in an exicator and cooled before the weight was taken anew. For gravimetric calculations the equation below was used. After weighing, the dried samples were pulverized and used in the following characterization analysis.

$$\%\ moisture = \frac{total\ weight - final\ weigth}{sample\ weight} \times 100$$

Protein content

The protein content of the different waste fractions was calculated based on the Dumas method, also known as the C-N (Combustion-Nitrogen)-method and can be used to rapidly measure the protein content in food. The method determines the total nitrogen content, which ultimately can be converted to protein when using conversion factors (Müller, 2017). Dried samples obtained when calculating moisture content was weighed accurately in small tin containers. The analysis was performed at CFS (Central Instruments Facility and Services) at CFTRI by fellow researcher Tharak Trivans.

Ash content

Ash content was measured by the AOAC method (AOAC, 1995). Empty crucibles were weighed and noted for gravimetric calculations. The analysis was run in duplicates, and between 0.5 -1 g dried powder was added to each crucible and accurate weight noted. The content of the crucibles was burned until there was only ash left and placed in a Muffle furnace at 550 °C for five hours. After five hours the oven was turned off, but the samples were kept overnight. The samples were collected in an exicator and left to cool before weight was measured anew. Using the equation below the percentage of ash in the raw material was calculated.

$$\%\, ash = \frac{final\ weight\ crucible - empty\ weight\ crucible}{sample\ weight} \times 100$$

Fat content

To measure the total fat content the Soxhlet method was used. The method was invented in 1879 by scientist von Soxhlet and is a well-known extraction method. In conventional Soxhlet the sample is placed inside an extraction thimble holder that is increasingly filled with condensed solvent from a distillation flask. Once the liquid reaches a set level, the whole thimble is emptied, and the solvent is transferred back to its distillation flask. The extracted analytes are kept in the round bottom flask at the heating mantle. The solvent is recirculated, and the process is repeated until the extraction is completed (Luque de Castro and García Ayuso, 2000).

Three round bottom flasks were weighed and noted. Between 0.5 -1 g of dried sample were accurately weighed onto filter paper and packed neatly. The samples were placed in a thimble holder of a Soxhlet extractor for 6 hours at 60 °C until the lipids were extracted. The round bottom flasks were placed in a heating oven at 105 °C overnight for the liquid to vaporize and only the lipid remaining in the flask. The following day the flasks were weighed for gravimetric calculations and the equation below was used to calculate the total fat content.

$$\%\, fat = \frac{final\ weight\ round\ bottom\ flask - empty\ weight\ round\ bottom\ flask}{sample\ weight} \times 100$$

3.2.2. "Ideal process" of enzymatic hydrolysis

The rest raw material received from Kaiko was intact. Enzymatic hydrolysis was performed to produce FPH that eventually would be further analyzed. Three types of rest raw material were received, namely head and viscera (HV), skin and bone (SB) and refined waste (RW). A total of 8 kg was received in several, smaller sample bags.

An "ideal process" of performing enzymatic hydrolysis in Norway and in general scientific literature on fish hydrolysates was outlined. The outline was based on important steps found in literature, from discussions with supervisors and lectures. The "ideal process" was brought to India to see if it could be used when conducting the enzymatic hydrolysis on raw material from Kaiko. The process is displayed in figure 10 with key elements underneath each segment. In order to produce high quality FPH it is of great importance that each of the key elements are maintained throughout the process. If one or several of the elements are not upheld it will affect the final quality of the FPH or if even possible to perform.

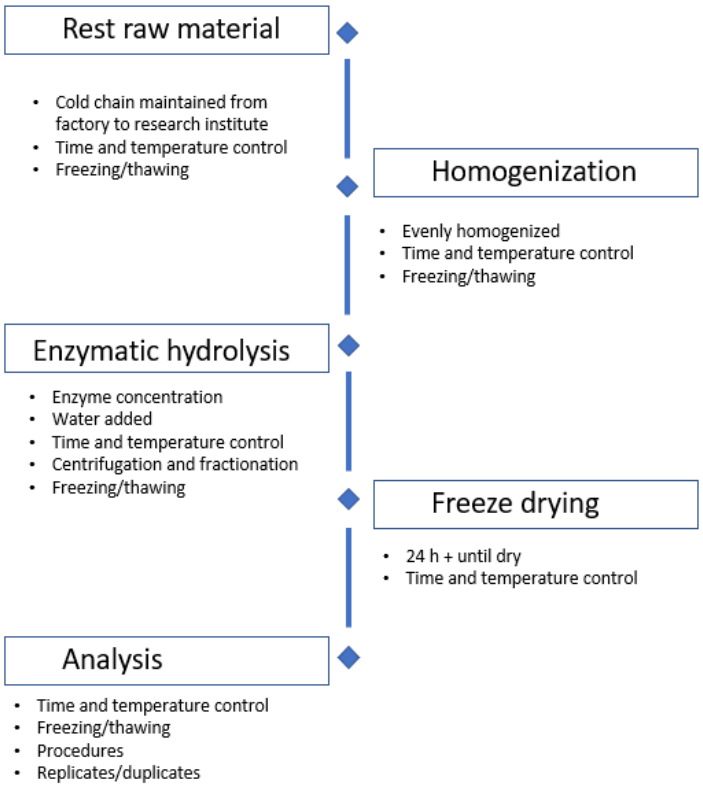

Figure 10: Ideal process of running an enzymatic hydrolysis in Norway. The goal is to perform this process in India and highlight the challenging aspects of the process

Firstly, one bag of each fraction from Kaiko was thawed and homogenized. The raw material was thawed in water baths for approximately 90 minutes and minced with water using a vertical cutter (robot coupe R 10 serie 5). A blender was used (Prestige PRO 250) to further homogenize the samples. This step was performed by Dr. Sachindra and a PhD candidate and we were only going to observe. Once the samples were thoroughly mixed, three samples of 50 g from each batch were placed in smaller plastic bags, while the

remaining samples were placed in bigger plastic bags before sealing and kept for frozen storage at -20 °C.

Before performing a large-scale enzymatic hydrolysis, the optimum conditions for each type of rest raw material was found through a small-scale hydrolysis. An experimental setup designed by Dr. Sachindra was followed as shown in table 3.

Table 3: Experimental setup to determine optimum conditions for enzymatic hydrolysis

	Temperature (℃)	Time (min)	Enzyme (% of raw material)
1	30	30	0.5
2		30	1.5
3		30	2.5
4		90	0.5
5		90	1.5
6		90	2.5
7		150	0.5
8		150	1.5
9		150	2.5
10	45	30	0.5
11		30	1.5
12		30	2.5
13		90	0.5
14		90	1.5
15		90	2.5
16		150	0.5
17		150	1.5
18		150	2.5
19	60	30	0.5
20		30	1.5
21		30	2.5
22		90	0.5
23		90	1.5
24		90	2.5
25		150	0.5
26		150	1.5
27		150	2.5

A total of 27 tests was performed, plus one blank test without enzyme for each of the temperatures. In total 10 conical flasks were used for each temperature. One of the 50 g bags were thawed in water bath and 40 g sample was weighed in a beaker and mixed with 120 ml distilled water (ratio 1:4). Then 10 ml was transferred into 10 different conical flasks, before adding different concentration of enzyme (Alcalase, *Bacillus licheniformis,* LOT: 2920868, Merck, Germany) except of one conical flask with no enzyme. The conical flasks were gently mixed, covered with aluminum foil and incubated at 30 °C, 45 °C and 60 °C for 30, 90 and 150 minutes, respectively. The incubator

(Pisces Reevo SL 3000, India) had a rotary function to keep the samples stirring. For the samples without added enzymes 2 ml was removed at each time interval. After incubation, 1 ml sample was transferred to a microcentrifuge-tube (mct) and 1 ml 5 % Trichloroacetic acid (TCA) was added for inactivation. These samples together with samples without enzymes were centrifuged (Sigma 1-14 K, 20 min, 4500 x g). The supernatant was collected in a new mct and frozen at -20 °C. The hydrolysate remaining in the conical flasks were transferred to 15 ml centrifuge tubes and placed in water bath at 90 °C for 15 minutes to inactivate the enzymes. Once inactivation was done, the samples were centrifuged (Eppendorf AG 22331, 5805, Hamburg, Germany 10 minutes, 8800 x g)

The degree of hydrolysis (%DH) was measured by using the trinitro-benzene-sulfonic acid (TNBS) method. This was performed by fellow master student Frida Holm Larsen, where these results were plotted in the software STATISTIKA and response surface methodology was used to visualize the optimum conditions. A complete description of this procedure can be found in Frida Holm Larsen's thesis.

After the optimum conditions was found, enzymatic hydrolysis in a larger scale was performed following the same procedure with those exact conditions. Initially it was decided to use 1 kg raw material and 3 L water. After the hydrolysis was finished the samples were centrifuged to separate the oil and sediments, and the supernatant was collected and prepared for freeze drying (Christ Gamma 2-16 LSCplus). Freeze dried samples were vacuum-packed and brought to Norway.

Questionnaire rest raw material India

3.3. SWOT-analysis

In terms of health, safety and environment (HSE), our university in Norway and our host institute in India, differed and have different laws and regulations in place that must be followed. To a certain extent, this created a few challenges in the execution of the planned analyses. With shortage of raw material, difficulties creating new hydrolysates and concerns regarding the safety in the lab, the course of the thesis was readjusted. To take advantage of the opportunity to gain more insight during our stay a questionnaire was created in cooperation with Norwegian supervisors on utilization of rest raw material in India. It was sent to several Indian professors and students at different institutes and universities as well as Indian industry to get different perspectives on the manner. The questionnaire was sent to Dr. Sachindra, Dr. Tumaney, Dr. Kudre and Dr. Rathina at CFTRI along with a few PhD candidates. The questionnaire was also sent to Dr. Nutan at Amity University and her students, as well as to Dr. Souvik at BITS Pilani whom are also participants in the ReFood project. To get insight from the industry we had a FaceTime conversation with daily manager of Kaiko, Asif Naik. Through this phone call we were given useful answers from an industrial perspective and got to try communication through technology rather than visiting the actual factory in Mumbai. The questionnaire was also sent to researchers at SINTEF Ocean that had participated in the workshop in Mumbai. Based on the answers gathered from the questionnaire and knowledge gathered from our own fieldwork and observational studies a SWOT-form (figure 11) was filled out.

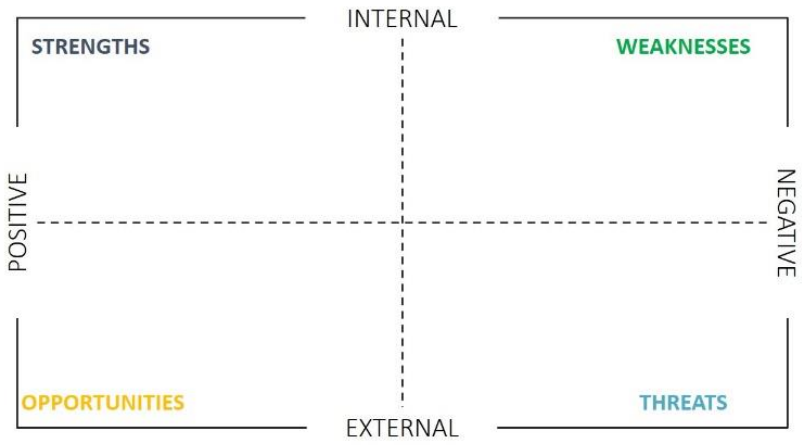

Figure 11: SWOT form with internal and external factors

4. Results and discussion

Fieldwork

All measures listed in table 1 attributed with valuable insight and useful knowledge in the outline of this study. By attending the workshop in Mumbai, an introduction to India and rest raw material utilization was given. The visit to surimi factory Kaiko with a tour of the factory gave general information on surimi production. Daily manager Asif Naik elaborated about the factory's challenges, and how the production could become more sustainable, and profitable through a collaboration within a research project such as ReFood.

Figure 12: A simple presentation of Kaikos production line from fish docking to finished surimi. (Pictures: Gupta, 2018 and private 2019)

A presentation of Kaikos production line is given in figure 12. Kaiko produces an estimate of 3000 tons surimi a year, and from this process 900 tons of rest raw material is generated in terms of heads, viscera, skin and bones, trimmings and refined waste. Today this material is either being used for production of fish meal or as fertilizer (Naik, 2019). Through the workshop in Mumbai acquaintances with Indian PhD candidates from Amity University was made, whom provided valuable information on Kaiko. In a report on Kaikos production by Gupta (2018), one of the PhD candidates, it was stated that from 1 ton of headless fish, only 45 % ends up as finished surimi products. In other words, tons of raw material are generated. In addition to this, huge amounts of process water (wastewater) from the washing steps in production goes to waste. This water is used to wash the meat, and naturally proteins and peptides are separated out and is poured down the drain together with the process water. Finding ways of collecting this water and

process it by membrane filtration could be a good way of retrieving lost proteins and is worth investigating. This was also one of the major topics of the workshop.

The workshop gave an opportunity for networking and to establish communication with the different Indian actors. At the business summit in New Delhi that connection was continued as several of the participants from the workshop also attended the summit. Amongst the participants were Dr. Nutan from Amity University, whom invited us to the university while in Delhi for a tour and to catch up with the PhD candidates we had met previously.

From the visit to Pelagia in November 2018 insight into FPH production was obtained and an understanding of how much material is involved. High season of herring is from October/November-January, and in this period weekly batches of approximately 6000 tons are received at Pelagias factory (Vestengen, 2018). The utilization of rest raw material has developed further in Norway, and as seen by the numbers it is a large industry in Norway. Naturally, India has the potential to benefit from collaborations such as ReFood.

The workshops along with company visits were good tools to investigate both the "ideal process" and provided good insight for the SWOT presented in section 4.2.3.
The Norwegian Research Council have invested a lot of money in both INTPART and into the ReFood project. The Prime Minister of Norway participating in the business summit on solutions for sustainable growth confirms the importance of international collaborations, knowledge exchange, and helps to understand the magnitude of these projects.

Laboratory work

When discussing the results obtained from the laboratory work, it is important to recall that the two cases, herring and surimi, are based on different foundations (figure 6). Raw material from Pelagia was industrial and analyzed directly, whereas on raw material from Kaiko enzymatic hydrolysis was performed to produce FPH prior to analyzing. As the samples from Pelagia already was processed, quality analysis of rest raw material prior to processing has not been performed. Pelagia informed that the fat content of the hydrolysates would be around 0.3 %. Findings from both fieldwork and laboratory work through observations are used as a basis in a SWOT-analysis which is presented and discussed last.

4.1. Herring

Crude oil

4.1.1. Thin layer chromatography

Two analyses were run on crude oil: TLC and fatty acid composition. In figure 13 the results from TLC is displayed with increasing concentrations of crude oil. Crude oil was dissolved in $CHCl_3$ and for sample H1 5 µg was placed on the TLC plate and for sample H2 10 µg was placed.

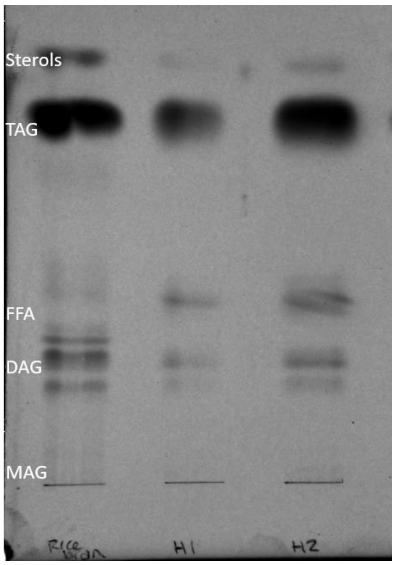

Figure 13: TLC of crude oil from herring (sample H1 and H2 with different concentrations of crude oil). Rice bran oil was used as standard.

The results of TLC showed that the crude oil contained mostly TAG seen as these bands are more distinct. In addition to TAG, the bands indicate presence of DAG and MAG, and and a minimal presence of FFA. The upper bond also suggests that the crude oil contains sterols. In a report by Falch et al. (2006) on seasonal variations in herring and mackarel, they found that the lipids in herring mostly consisted of TAG (89-93 %). This corresponds with TAG displaying as the most distinct bands. Their results showed that the highest content of TAG in herring was in october-november which coninsides well as the crude oil was produced in November. However, these results are based on herring filet and not crude oil, and thus are not completely comparable.

It was attempted to create a standard curve for TLC. The final curve was not accurate enough to be used and quantification was not possible. The TLC plate still provides a good visualization of what is present in the crude oil. It is important to note that different solvent system are used for separation of triacylglycerides and phospholipids, and thus several TLCs should have been performed on crude oil. If possible, it would have been optimal to run lipid classes with a more accurate method than the one used. High resolution nuclear magnetic resonance spectroscopy (NMR) could also have been used if the HSE conditions had been in accordance with NTNUs requirements.

4.1.2. Fatty acid composition of crude oil

The FA composition of herring crude oil is given in table 4. Raw data can be found in appendix A.

Table 4: Fatty acid composition of crude oil Pelagia

Fatty acid	*mg/g*	*% of total FA*	*SD*
C14:0	98.28 ± 1.20	9.95	0.16
C14:1	3.26 ± 0.04	0.33	0.00
C15:0	5.58 ± 0.06	0.56	0.01
C16:0	137.20 ± 0.67	13.89	0.12
C16:1 n 9	58.28 ± 1.93	5.90	0.17
C17:0	5.34 ± 0.04	0.54	0.00
C17:1	3.09 ± 0	0.31	0.00
C18:0	11.42 ± 0.50	1.16	0.06
C18:1n11	96.10 ± 0.11	9.73	0.03
C18:1n7	14.71 ± 0.02	1.49	0.00
C18:2n6	12.15 ± 0.04	1.23	0.00
C18:3n6	1.86 ± 0.27	0.19	0.03
C18:3n3	9.17 ± 0.01	0.93	0.00
C18:4n3	25.03 ± 0.21	2.53	0.03
C20:0	1.54 ± 0.04	0.16	0.00
C20:1	147.78 ± 1.63	14.96	0.11
C20:2n6	1.81 ± 0.06	0.18	0.01
C20:3n6	0.68 ± 0.01	0.07	0.00
C20:4n6	1.81 ± 0.07	0.18	0.01
C20:3n3	1.01 ± 0.02	0.10	0.00
C20:4n3	4.56 ± 0.01	0.46	0.00
C20:5n3	**67.31 ± 0.48**	**6.81**	**0.08**
C22:0	0.13 ± 0.04	0.01	0.00
C22:1n11	188.86 ± 3.50	19.12	0.28
C22:1n9	10.32 ± 0	1.04	0.00
C22:2	2.98 ± 0.01	0.30	0.00
C22:3	0.75 ± 0.23	0.08	0.02
C22:4	1.07 ± 0.01	0.11	0.00
C22:5n3	6.76 ± 0.04	0.68	0.00
C24:0	0.00	0.00	0.00
C22:6n3	**62.54 ± 0.50**	**6.33**	**0.08**
C24:1n9	6.53 ± 0.23	0.66	0.02
TOTAL	987.825	100.00	

The percentage of total FA shows an EPA content of 6.81 % and 6.33 % of DHA and are highlighted in table 4. This is a rather high content of omega-3 LC-PUFAs, and despite only one batch of crude oil was received and seasonal variations cannot be investigated, it is observed that the results do correspond with expected lipid content in herring from November of previously research. (Falch et al., 2006, Carvajal et al., 2015).

Fish protein hydrolysates

4.1.3. Fractionation of FPH by membrane filtration

Three different fractions were obtained through ultrafiltration. The different fractions obtained were retentate (150 kDa), retentate (4 kDa) and permeate (4 kDa).

Figure 14: All fractions of FPH (freeze-dried after filtration) From left to right: original FPH (A), retentate 150 kDa (B), retentate 4 kDa (C) and permeate 4 kDa (D)

The fractionation aimed to sort the proteins and lipids by size, and to remove impurities through the clarification process with the ceramic membrane. As observed from figure 14, all fractions showed differences in both color and texture, with fraction C and D being of a gooier texture.

Unfortunately, the flux value was not noted. Neither was the amount of permeate retrieved. In hindsight this would have been valuable information that could have provided information to calculate mass balances. All four fractions were brought to India to be used by all three master students. This led to some uncertainties as to if it would be enough sample material to run analyses.

4.1.4. Total lipid content of FPH

The result from the Bligh & Dyer method are presented in table 5 and shows the total lipid content of each of the fractions from Pelagia. See appendix B for raw data.

Table 5: % lipid content in FPH fractions from Pelagia stated as % of dry matter. Fraction A (average, n=6, SD). Fraction B-D (value, n=1)

Sample	*% lipid*
Fraction A (original FPH)	13.8 ± 0.29
Fraction B (retentate 150 kDa)	20.54
Fraction C (retentate 4 kDa)	1.13
Fraction D (permeate 4 kDa)	0.49

The total lipid content of the original hydrolysate was 13.8 % on a dried weight basis. The high lipid content in fraction A (original FPH) was not expected as it was claimed by Pelagia that the fat content was around 0.3 % (w/w). It has previously been suggested

by Spinelli et al. (1972) that the lipid content of FPH should not exceed 0.5 % as it becomes less stable and hard to prevent lipid alterations during storage. The high lipid content of the original hydrolysate might indicate difficulties in the separating process prior to hydrolysis (figure 3), and perhaps the process conditions are not optimal. Phospholipids have strong emulsifying properties that could cause problems in separation of the oil (Frankel, 2014).

The highest lipid concentration is found in fraction B (retentate 150 kDa) with 20.54 %, and the lipid content decreases sharply in the following two fractions with only 1.13 % and 0.49 %. It is likely that phospholipids are retained in fraction B (retentate 150 kDa) due to their size. The fat content of fraction C and D is significantly lower and may suggest that they are not as susceptible for lipid oxidation. However, this must be considered together with FA composition whether the fat consists of PUFAs that gets oxidized easily.

4.1.5. Separation of lipid classes

It was possible to separate both neutral lipids, glycolipids and phospholipids, but the quantity was very low. The quantity obtained from each fraction was enough to perform TLC, although it was only time to perform TLC on fraction B (retentate 150 kDa) which gave poor developed plates, and the plate for glycolipids was destroyed during charring and is not presented. TLC for neutral and phospholipids are shown in figure 15. The plate of neutral lipids did not develop properly, whereas for phospholipids it appears that the separation has not been successful. Phospholipids show distinct bands in the second row (oil sample) but in the fourth row (separated phospholipids) nothing is displayed. TLC was performed on fraction B prior to fraction A (original FPH) due to the solid phase extraction of fraction A had to be repeated. The results from solid phase extraction were deemed inconclusive as they would have to be repeated. Due to the HSE situation this was not possible.

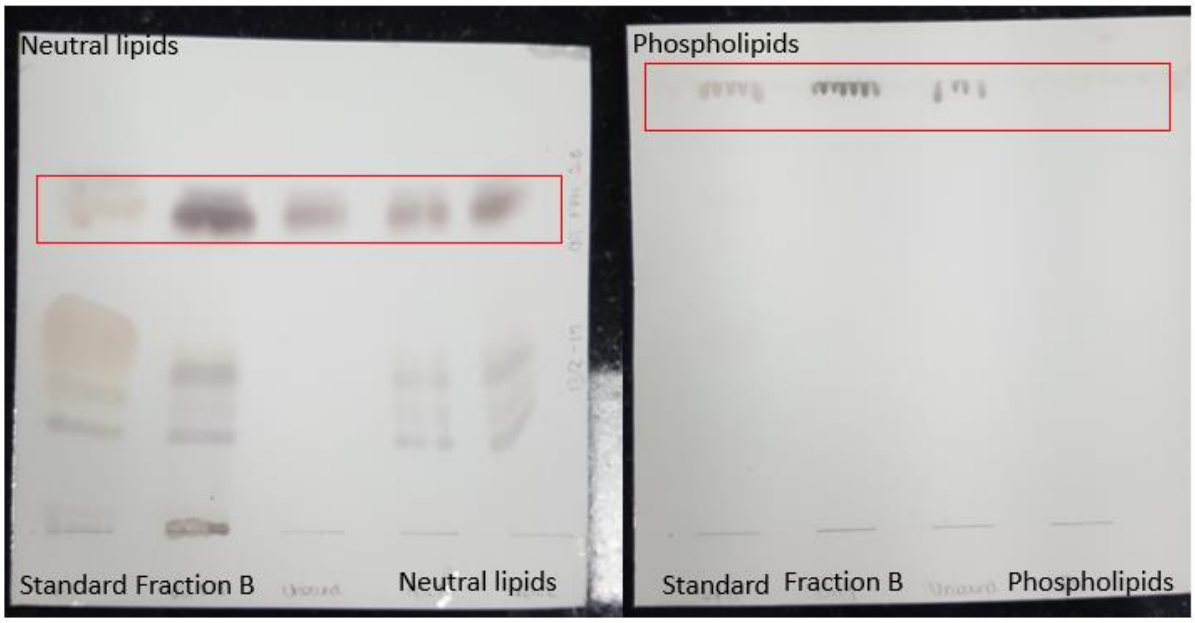

Figure 15: TLC plates of neutral lipids and phospholipids from fraction B (retentate 150 kDa). Left: the plate for neutral lipids did not develop correctly, and the standard was not sufficiently diluted. Red box indicates TAG. Right: There are clear bands of phospholipids present in the oil, but do not appear for the separated phospholipids. Red box indicates phospholipids.

4.1.6. Fatty acid composition of FPH

GC-FID was run on the herring oil extracted from fraction A (original FPH) from Pelagia. Despite GC only was run on one fraction it gave a general good FA profile of the FPH, as all other fractions are derived from this one.

Table 6: Composition of fatty acids (percentage of total fatty acids) in extracted oil from fraction A (Pelagia)

Fatty acid	µg FA*	% FA of total FA
C14:0	48.30	13.98
C15:0	1.96	0.57
C16:0	77.01	22.29
C18:1	5.98	1.73
C18:1 (trans 13)	38.05	11.01
C18:1 (trans 13)	5.96	1.73
C20:2	4.21	1.22
C18:4	6.60	1.91
C20:1	52.24	15.12
C22:1	53.30	15.43
C20:5	**25.97**	**7.52**
C22:6	**25.94**	**7.51**
TOTAL	345.52	100.00

*µg FA Could not determine the proper unit to present µg of.

The results are presented as % of total fatty acids

In table 6 the FA composition is presented in the order of the retention time of the peaks (appendix C). The amount of EPA and DHA of the total FA were 7.52 % (25.97 µg) and 7.51 % (25.94 µg), respectively. In an article by Carvajal et al. (2015) on producing high quality oil from herring by-products, they found higher amounts of both EPA and DHA where the total of FA was 7.7 % (71.1 mg/g) and 10.2 % (92.4 mg/g), respectively. Additionally, there was a 2.5 % higher level of DHA than EPA in their FA profile, whereas the amount of EPA and DHA differ with 0.01 % in the results obtained in India. However, the amount still shows a good omega-3 content and a high content of MUFAs, especially C20:1 and C22:1. This is coherent with the report from Falch et al. (2006) on seasonal variations in herring and mackerel, whom also found that C20:1 and C22:1 constituted the majority of the FA and also had a high omega-3 content.

The results from the GC itself was a bit unclear as the sequence of the peaks were a bit abnormal. A GS-MS (Gas chromatography mass spectroscopy) was run to clarify the results. Typically, the peaks should appear in an ascending order, and in this case C20:2 appeared prior to both C18:4 and C20:1. No apparent reason was found for this, other than the set default program is normally for vegetable oil and not oils of marine origin. Due to shortage of material it was not possible to run replicates, which argue that the results have a level of uncertainty as neither SD nor any other statistical analysis could be run. It would be preferable to verify the data through more accredited methods.

4.2. Surimi

4.2.1. Characterization of raw material

The different fractions received from Kaiko are displayed in figure 16. As shown, head and viscera consisted of mostly head and refined waste consisted of muscle and connective tissue. In total 8 kg was sent from Kaiko, 3 kg HV, 3 kg SB and 2 kg RW.

Figure 16: Photos of rest raw material received from Kaiko taken after thawing prior to homogenizing. All three fractions from the right: head and viscera (HV), skin and bone (SB) and refined waste (RW) (Photos: Larsen, 2019)

Table 7: Characterization of rest raw material from Kaiko**.** Moisture, protein, fat and ash stated as percentage of w/w. Moisture and ash (average ± SD, n=2), protein and fat (value, n=1) HV= Head and viscera waste, SB= Skin and bone waste, RW: Refined waste.

	Moisture content (%)	*Protein (%)*	*Fat (%)*	*Ash (%)*
Fraction A: HV	78.58 ± 0.14	12.48	1.44	7.04 ± 0.44
Fraction B: SB	82.67 ± 0.94	9.46	0.78	7.29 ± 0.73
Fraction C: RW	84.59 ± 0.37	12.07	0.54	2.96 ± 0.37

In table 7 data from the characterization of the raw material is presented as percentage of wet weight. The moisture content of the different fractions was rather similar. The fat content is highest in the HV fraction. This is expected as the viscera, and particularly the liver, are found in this fraction. However, as shown in figure 16 the HV fraction mostly consist of heads. As mentioned in section 3.2.1 Kaiko uses a decapitating machine that removes head and viscera in one step. It was possible to observe that viscera were present but the ratio between heads and viscera is uneven, and there is an uneven distribution of the viscera in the material received. Based on this the fat content will vary, and the fat content of 1.44 % is not representative for the remaining material. The protein content is relatively higher in the RW fraction. This is expected as this fraction mostly consists of muscles and connective tissue. However, when measuring fat and protein content duplicates was not used. Thus, standard deviation (SD) could not be calculated and the results have uncertainties.

4.2.2. ”Ideal process” of enzymatic hydrolysis

The enzymatic hydrolysis was, as previously stated in section 3.2.2, performed multiple times. First, the optimum conditions had to be found before a large-scale hydrolysis could be performed with those conditions.

The experimental set-up displayed in table 3 was followed to find optimum conditions. Samples were thawed in water bath without any form of temperature control and the amount of water added during homogenization is not known. It was not possible to get the raw material completely homogenized, likely because of the material’s natural inhomogeneity (skin, head, bones etc.). After mixing the now “homogeneous” rest raw material with water (1:4), it was transferred to the 10 different conical flasks. However, the material was not evenly distributed between the flasks and chunks of varying size in the conical flasks (figure 17, left) is shown. This indicates that there is a variation in the ratio between enzymes and substrate in each of the flasks, and thus the reaction rate within them may differ. The hydrolysis was not performed with accuracy and there were discrepancies between the conical flasks, in addition to little temperature control. In hindsight the samples should have been mixed directly in the conical flasks to ensure even distribution. The degree of hydrolysis that ultimately gave the optimum conditions may be incorrect.

Figure 17: Steps in performing enzymatic hydrolysis at CFTRI Left: one of the conical flasks before enzyme was added displaying chunks of raw material. Right: After hydrolysis (Photos: Larsen, 2019)

After the optimum conditions were found there was only enough time to run hydrolysis for one fraction, seen as it took an entire day to complete the hydrolysis for one fraction. The HV fraction had the highest lipid content, making it optimal to proceed with this fraction for all three students. Thus, the other fractions were no longer being used and will not be presented further in this thesis. For HV the optimum conditions were found to be 30 °C, 30 minutes and 0.5 % enzyme. This was not expected, seen as the optimum temperature of Alcalase is between 50-60 °C. (Aluko, 2018) It was discussed if it was better to proceed with known optimum conditions for Alcalase or continue with the results obtained. After discussing this with Dr. Sachindra we decided to use the optimum conditions found from %DH to run the large-scale hydrolysis. However, we found it difficult to trust the results, and this consequently remains an uncertainty.

Two 2-liter beakers were used (figure 17, right) when producing the big batch. This caused some troubles during incubation, as the samples were too heavy, and the rotary function did not work. The rotary function was replaced by occasional manual stirring, which meant the incubator was opened and the temperature did not remain at 30 °C. The optimum conditions were not upheld, and on an industrial level this would be a huge problem as everything has to be produced under controlled circumstances.

After inactivation the hydrolysates had to be centrifuged. The centrifuge (Eppendorf AG 22331, 5805, Hamburg, Germany) had a maximum capacity of 300 ml a time, and we had 4 liters. It was recommended to use a separator (figure 18) instead, but this did not work as it was leaking, did not separate the oil and was not clean. This was performed in a pilot plant by a technician. Eventually we stopped the procedure and the hydrolysate had likely been contaminated. The quality of the hydrolysate became questionable and it was not favorable to continue with this batch as it would become an uncertainty in later analyses.

Figure 18: Separator used to remove the oil (Photo: Larsen, 2019)

A new batch was made from the HV fraction. However, at this time the departure date was coming up and the freeze-drier was fully booked two weeks ahead. A slot became available – but only for one liter. After discussing with both Dr. Sachindra and our supervisors in Norway the ratio was changed to 1:1. 500 g HV and 0.5 L water was used to get as high yield as possible. This ratio is more commonly used in the literature, and in articles by Opheim et al. (2015) and Slizyte et al. (2016) this ratio was used when performing enzymatic hydrolysis. From an industrial and economical point of view it would be desirable to keep a low water content. Less water to heat and eventually dry would result in a reduction of expenses.

During centrifugation it was not possible to separate the oil from the aqueous phase. It was believed that this was due to a low capacity of the available centrifuge. After increasing the g-force the results were the same. This indicates that there is little oil present. This coincides with what was found in section 4.2.1, that the HV fraction consists mostly of heads and that the viscera are unevenly distributed in the raw material. Only the supernatant (hydrolysates) were freeze dried, while sediments had to be dried in a heating oven at 105 °C. This was not favorable, as the high temperature may break down the material. Freeze drying gave approximately 40 g yield of FPH, while the heating oven gave 35 g of sediments.

After numerous attempts of performing an enzymatic hydrolysis at CFTRI it became evident that there were several challenges in the "ideal process". Together with observations from fieldwork the three most challenging aspects were found and is highlighted in figure 19.

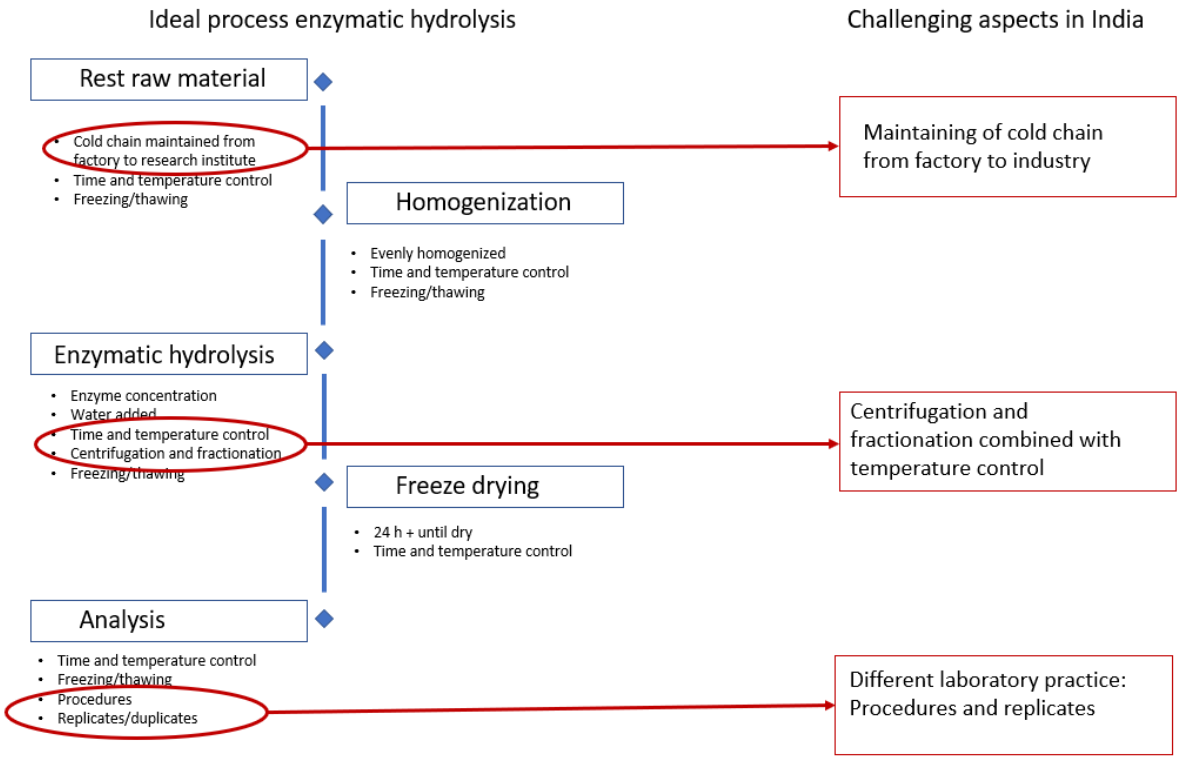

Figure 19: Representation of the three most challenging aspects when performing an enzymatic hydrolysis in India with Norwegian standards, based on both laboratory work and information gathered from fieldwork.

The first aspect is a step *prior* to the hydrolysis. There were a few challenges in transporting the raw material from Kaiko in Mumbai to CFTRI in Mysore. The main issue being maintaining the cold chain from the factory to the institute. Vehicles without cooling equipment makes it difficult and might contribute to more rest raw material being discarded. In figure 20 a vehicle for transportation of rest raw material at Kaiko is shown. Discarded rest raw material from the factory is placed inside and transported without any form of cooling equipment. The material transported this way is not to be utilized. This is a good example of how the infrastructure in India differs from Norway. The high temperatures in India makes the process more challenging as the material is more exposed to heat, and thus deterioration and oxidation. The rest raw material we received was frozen upon reception, but whether the cold chain was broken along the way is not known and present a valid concern.

Figure 20: Photos of vehicle for transportation of rest raw material from Kaiko. The rest raw material transported in this vehicle is waste and will be discarded. No cooling equipment present (Photos: Falch, 2018)

The second aspect is regarding the centrifugation and overall lack of temperature control. The issues with centrifugation can be explained due to a low fat content of the material, but equipment of old age could be a contributing cause. Temperature control was not upheld during hydrolysis making the %DH uncertain. Temperature control is important to avoid microbial growth and general deterioration of the raw material. These products were meant for human consumption after further processing and maintaining a good temperature control is important to maintain good quality and safe products.

The last aspect is regarding the lack of set procedures and a different overall laboratory practice. To our knowledge, there were often no procedures available in the lab, and the procedures that were made available to us often did not require replicates. Thus, on several occasions it was decided to not use replicates when running analyses, which also have been seen from the other analytical work. This is a major uncertainty that must be considered when discussing the credibility of the overall results obtained from these experiments. CFTRI is a recognized research institute, although regarding this sort of experiment (production of FPH) it seemed their experience were based on other materials rather than highly susceptible marine rest raw material. Additionally, it seemed like their previous experience were based on more homogenous materials. Marine rest raw material, such as skin and bones, are highly inhomogeneous and likely to affect the results. Lastly, the available process equipment was not regarded as satisfactory for the purpose of performing enzymatic hydrolysis. These limitations prevented us from performing "the ideal process" but helped us all in gaining major experience in where the critical points are in the process and how do approach them in later studies.

4.3. SWOT-analysis

The planned laboratory work did not go according to plan and was stopped midway into our exchange due to the differences in HSE between our university and our host institute. As described in section 3.3 a SWOT-analysis was conducted with the basis of a questionnaire regarding the potential of rest raw material utilization in India and our own personal experience gained through observation, participation, workshops and company visits. In this case the SWOT is used to identify the strengths, weaknesses, opportunities and threats to the potential of rest raw material utilization in India. Only a few

respondents participated, namely Dr. Sachindra, Dr. Nutan, daily manager Asif Naik at Kaiko along with a few from SINTEF Ocean. The answers obtained from the questionnaire along with our own findings from the fieldwork is presented in a SWOT (figure 21) and are what we have highlighted as the most important discoveries. When discussing the results of the SWOT it is important keep in mind that these are based on the answers of a few respondents – and does not represent the majority of India and is not an authentic representation of today's situation in India. The discussion is entirely based on the answers given by the respondents along with own observations and is not scientifically based.

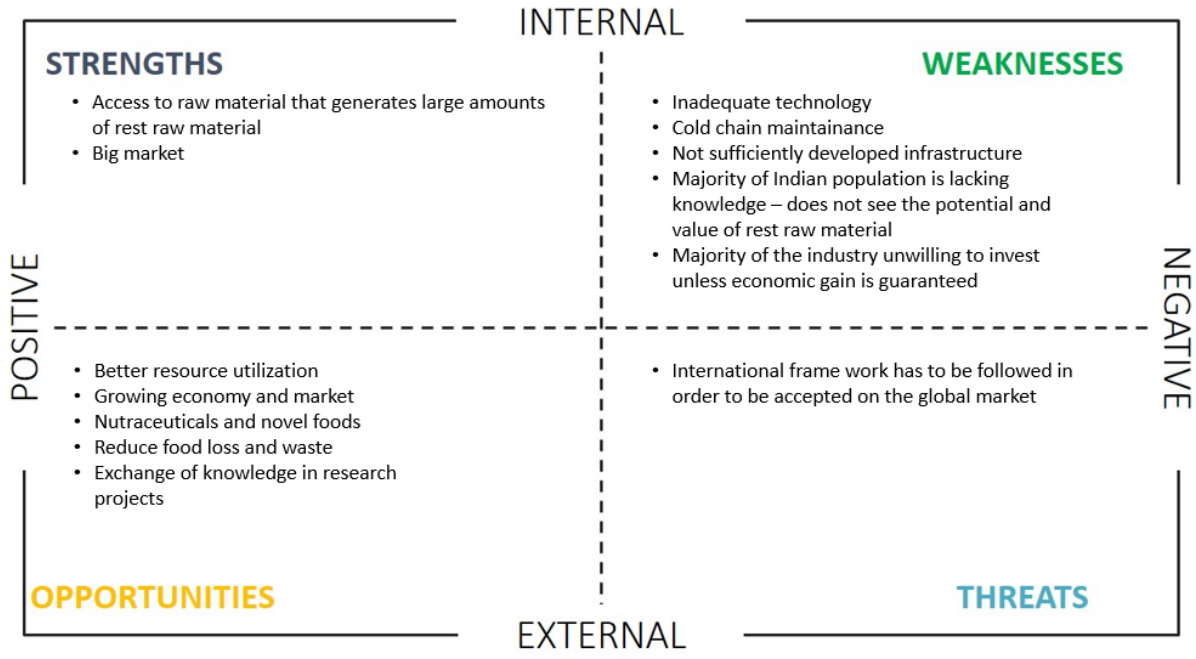

Figure 21: SWOT analysis based on questionnaire regarding potential of marine rest raw material utilization in India and own observations

Weaknesses and threats

India is situated by the Indian Ocean with good access to marine landings. This naturally provides an abundance of raw material which in turn generates large amounts of rest raw material from the marine industry. Although, when it comes to utilizing these resources there are a few challenges. Perhaps the biggest weakness lies in an underdeveloped infrastructure along with inadequate technology. Without necessary technology and proper processing techniques the rest raw material remains an underutilized resource and is used for low-value production or even discarded as waste.

The underdeveloped infrastructure creates challenges in the handling of rest raw material, as the distances between where the rest raw material is generated and the processing facilities can be far. For instance, at one point we visited a local fish market and got to observe how and what they do with the rest raw material they are generating. This was a small shop and the quantity they produced was so low it was discarded. The cost of keeping it refrigerated until collected would be greater than the potential reward. The infrastructure is, as of today, not developed to handle this sort of problems. By developing the infrastructure according to international standards regarding

transportation, processing facilities and landing sites it would improve the potential for rest raw material utilization. Another issue that occurs from lack of infrastructure is the maintainance of the cold chain. For highly perishable products such as rest raw material it is eminent that the cold chain is kept intact to ensure safe products of high quality. This becomes increasingly important if the goal is to produce products for human consumption.

Another weakness that was found was that the general knowledge on rest raw material in India seemed to be rather low and neither potential nor the value of it was recognized. This goes hand in hand with an unwillingness to invest unless profit is guaranteed. If people knew it was beneficial to make use of "the waste" they might be willing to invest. By enlightening people from a young age that everything has value it could prompt change and make way for new opportunities.

Strengths and opportunities.
The large amount of rest raw material that is generated yield opportunities as well. One of the challenges were the big distances between processing facilities and the origin of rest raw material. By building several small-scale processing facilities closer to where the rest raw material is generated it would improve the utilization rate, and thus lead to a reduction in food loss and waste. Today most of the rest raw material today is either being used as fertilizer or production of fish meal or discarded. Small-scale facilities might improve the possibilities of producing either novel foods or nutraceuticals meant for human consumption. However, that indicates that both Indian and international frameworks must be followed for it to become sellable, legitimate products and could in turn become a new challenge.

International collaborations contribute with knowledge exchange, and Mr. Asif Naik was very positive to participate in research projects such as ReFood and ReValue. The opportunity to learn from researchers and be able to develop the factory to become more profitable yet also more sustainable was appreciated. In the FaceTime dialogue with Mr. Naik it was discussed if he could guide us through the factory as if we had been in Norway. It was desirable to use technology to observe and get a glimpse of the factory, but the technology needed for such interactions were not yet present at the factory. It seemed like the biggest issue was lack of internet access in the factory. This naturally presents itself as an opportunity for the future, although remains a weakness for the time being. If the technology was implemented, it would be possible to establish an active dialogue and contribute in the development of the factory in India while working from Norway. This would have had several benefits and could be environmentally beneficial by e.g. reducing amount of airplane travels once such a relationship is established.

Final reflections

At the beginning of the project the initial plan was to conduct all the analytical work at CFTRI. The plan was to look at differences between Norway and India with focus on both lipid analyses and enzymatic hydrolysis. I was to perform the lipid analyses that were available in India, and look at differences in execution, and perform the same analyses on the FPH we would produce from surimi. Around halfway into our exchange it was decided together with our supervisors that the HSE regulations practiced at the institute was not in accordance with those of NTNU. With minimal use of fume hoods and different laboratory practice it was regarded unsafe for us to finish and continue working on the different analyses we had begun. No analyses are more important than one's health, and even though it was suggested that my Indian peers in the lab could finish the analyses for me, it did not seem ethical. Based on this the focus of the thesis was readjusted. The results that we had been able to obtain in the lab was still of value, but valuable information through qualitative analytical methods was also gathered on challenges and potential of rest raw material utilization in India. Through our own observations we learned what one might have to expect when travelling to a completely different part of the world. Things that are taken for granted in Norway might not be common other places and is an important lesson to keep in mind when doing research abroad.

5. Conclusion

This thesis has been rather atypical, and it seems natural to divide the conclusion into two parts. The main findings were those obtained from a fieldwork perspective, as the results obtained from analyses in the lab is highly characterized by uncertainties and would benefit from being validated through repeated analyses.

Fieldwork
Results obtained from fieldwork combined with practical work showed that there were several challenging aspects in performing a predefined "ideal process" of enzymatic hydrolysis in India. One of the challenges was the maintaining of cold chain from factory to destination, along with differences in laboratory practice. In addition, the rest raw material showed little homogeneity and was a challenging material to work with. In addition, it is a highly perishable raw material, which emphasizes the importance of a well maintained cold chain. Results from the SWOT-analysis showed a big potential for utilization of rest raw material in India, and that an underdeveloped infrastructure along with little knowledge on the manner creates difficulties for an optimal utilization.

Laboratory work

The results from the lipid analyses on both crude oil and FPH from herring suggests that there is an overall high content of the health beneficial fatty acids EPA and DHA. In crude oil the EPA and DHA content was 6.81 % and 6.33 %, respectively. In FPH this was found to be 7.52 % EPA and 7.51 % DHA and is coherent with what the expected lipid content in November caught herring is when regarding seasonal variations. Although, replicates were not used and causes some skepticism to the findings. (% of total fatty acids)

The different fractions from ultrafiltration showed that fraction B (retentate 150 kDa) had the highest lipid concentration of 20.54 % and indicates that most of the phospholipids are retained in this fraction. This indicates that fraction C (retentate 4 kDa) and D (permeate 4 kDa) might be less susceptible to lipid oxidation, although it must be seen together with fatty acid composition to determine amount of polyunsaturated fatty acids.

Regarding the lipids of surimi rest raw material, the overall lipid content was very low. The highest content was found in the head and viscera fraction (1.44 % w/w). The material is unevenly distributed, and a challenge would be to ensure equal quantity each time.

6. Suggestions for further work

Newfound knowledge and insight into the utilization of marine rest raw material in India have been obtained through the ReFood project and exchange to India. Challenges in performing the predefined "ideal process" was uncovered that was not known prior to the exchange, and challenges that was not expected have appeared. Without the opportunity to do this exchange these challenges would not have been uncovered, and it is evident that this is important for future work on marine rest raw materials in India. Future work on the utilization of rest raw materials from surimi production can use this work as a basis for moving forward towards the goal of increased utilization of marine rest raw material.

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Appendix A

Raw data for fatty acid composition (mg/g) in crude oil (SINTEF Ocean)

Par.nr	1	2	avg	std
Fettsyre				
C14:0	97.43	99.13	98.28	1.20
C14:1	3.28	3.23	3.26	0.04
C15:0	5.54	5.62	5.58	0.06
C16:0	136.72	137.67	137.20	0.67
C16:1	59.64	56.91	58.28	1.93
C17:0	5.36	5.31	5.34	0.04
C17:1	3.09	3.09	3.09	0.00
C18:0	11.06	11.77	11.42	0.50
C18:1n11 +n9	96.18	96.02	96.10	0.11
C18:1n7	14.72	14.69	14.71	0.02
C18:2n6	12.17	12.12	12.15	0.04
C18:3n6	2.05	1.67	1.86	0.27
C18:3n3	9.16	9.17	9.17	0.01
c18:4n3	24.88	25.17	25.03	0.21
C20:0	1.57	1.51	1.54	0.04
C20:1	148.93	146.63	147.78	1.63
C20:2n6	1.85	1.76	1.81	0.06
c20:3n6	0.67	0.68	0.68	0.01
C20:4n6	1.76	1.86	1.81	0.07
C20:3n3	0.99	1.02	1.01	0.02
c20:4n3	4.55	4.56	4.56	0.01
C20:5n3	66.97	67.65	67.31	0.48
C22:0	0.10	0.16	0.13	0.04
c22:1n11	191.33	186.38	188.86	3.50
C22:1n9	10.32	10.32	10.32	0.00
C22:2	2.97	2.98	2.98	0.01
C22:3	0.59	0.91	0.75	0.23
C22:4	1.06	1.08	1.07	0.01
c22:5n3	6.79	6.73	6.76	0.04
C24:0	0.00	0.00	0.00	0.00
C22:6n3	62.18	62.89	62.54	0.50
C24:1n9	6.69	6.36	6.53	0.23
sum	990.60	985.05	987.83	3.92

Sum sat	257.78	261.17	259.48	2.40
Sum mono	534.18	523.63	528.91	7.46
Sum poly	198.64	200.25	199.45	1.14
sum omega 3	175.52	177.19		

Raw data for fatty acid composition in crude oil Pelagia (% of total FA) (SINTEF Ocean)

Par.nr	1	2	avg	std
Fettsyre				
C14:0	9.84	10.06	9.95	0.16
C14:1	0.33	0.33	0.33	0.00
C15:0	0.56	0.57	0.56	0.01
C16:0	13.80	13.98	13.89	0.12
C16:1 n 9	6.02	5.78	5.90	0.17
C17:0	0.54	0.54	0.54	0.00
C17:1	0.31	0.31	0.31	0.00
C18:0	1.12	1.19	1.16	0.06
C18:1n11	9.71	9.75	9.73	0.03
C18:1n7	1.49	1.49	1.49	0.00
C18:2n6	1.23	1.23	1.23	0.00
C18:3n6	0.21	0.17	0.19	0.03
C18:3n3	0.92	0.93	0.93	0.00
c18:4n3	2.51	2.56	2.53	0.03
C20:0	0.16	0.15	0.16	0.00
C20:1	15.03	14.89	14.96	0.11
C20:2n6	0.19	0.18	0.18	0.01
c20:3n6	0.07	0.07	0.07	0.00
C20:4n6	0.18	0.19	0.18	0.01
C20:3n3	0.10	0.10	0.10	0.00
c20:4n3	0.46	0.46	0.46	0.00
C20:5n3	6.76	6.87	6.81	0.08
C22:0	0.01	0.02	0.01	0.00
c22:1n11	19.31	18.92	19.12	0.28
C22:1n9	1.04	1.05	1.04	0.00
C22:2	0.30	0.30	0.30	0.00
C22:3	0.06	0.09	0.08	0.02
C22:4	0.11	0.11	0.11	0.00
c22:5n3	0.69	0.68	0.68	0.00
C24:0	0.00	0.00	0.00	0.00
C22:6n3	6.28	6.38	6.33	0.08
C24:1n9	0.68	0.65	0.66	0.02
sum	100.00	100.00		

Sum sat	26.02	26.51	26.27	0.35
Sum mono	53.92	53.16	53.54	0.54
Sum poly	20.05	20.33	20.19	0.20
sum omega 3	17.72	17.99	17.85	0.19

Appendix B

Raw data for calculating total lipid content in FPH from Pelagia

B&D Fraction A (original FPH) Gløshaugen							
Samples	Weighed test tubes (g)	Veid fett etter avdamping i g	Differansen i g (a)	b	c	v	% lipid content
S1 I	13.9804	14.0139	0.0335	40	2	5.0167	13.36
S1 II	14.2116	14.2467	0.0351	40	2	5.0167	13.99
S2 I	14.2861	14.3219	0.0358	40	2	5.0548	14.16
S2 II	14.1145	14.1493	0.0348	40	2	5.0548	13.77
S3 I	14.4039	14.4389	0.035	40	2	5.0419	13.88
S3 II	14.3156	14.3499	0.0343	40	2	5.0419	13.61

Calculation % totalt lipid content			SD Fraction A	
13.80			0.29	

B&D Fraction B (retentate 150 kDa) CFTRI							
Sample	Initial weight	Final weight	Total weight in g (a)	Added CHCl3 ml (b)	Evaporated CHCl3 ml (c)	sample weight (v)	
FPH B	0.94273	1.04263	0.0999	4	4	0.4863	

Calculation % totalt lipid content	
20.54	

B&D Fraction C (retentate 4 kDa) CFTRI							
Sample	Initial weight	Final weight	Total weight in g (a)	Added CHCl3 ml (b)	Evaporated CHCl3 ml (c)	sample weight (v)	
FPH C	3.21963	3.22496	0.00533	4	4	0.4732	
Calculation % totalt lipid content							
1.13							

B&D Fraction D (permeate 4 kDa) CFTRI							
Sample	Initial weight	Final weight	Total weight in g (a)	Added CHCl3 ml (b)	Evaporated CHCl3 ml (c)	sample weight (v)	
FPH D	0.9816	0.9791	0.0025	4	4	0.5108	

Calculation % totalt lipid content		
0.49		

Appendix C

Raw data for determination of fatty acid composition FPH Pelagia

Peak #	Name	Area		µg FA
4	C14:0	9795163		48.2989868
6	C15:0	396792.3		1.95654386
7	C16:0	15617695		77.0093203
11	C17:0	10140133	Standard	
13	C18:1	1212836		5.98037521
15	C18:1 (trans 13)	7716258		38.0481104
16	C18:1 (trans 13)	1209437.1		5.96361557
17	C20:2	852853.1		4.20533488
19	C18:4	1337518.9		6.59517434
21	C20:1	10595310.00		52.2444331
22	C22:1	10809929		53.3026983
23	C20:5	5266923		25.9706801
25	C22:6	5260978		25.9413659

			Sum all FA	345.516639

Equation:

$$\mu g\ FA = \frac{area\ of\ unknown * 50\ \mu g\ internal\ standard}{area\ of\ internal\ standard}$$

Appendix D

Questionnaire on rest raw material utilization in India

1. How is rest raw material from the marine sector utilized in India today? Do you have any numbers of how much rest raw materials gets utilized and what goes to waste during one year?

2. What technologies and processes could be used for utilization of rest raw materials for value added products, especially regarding lipids and proteins?

3. Historically, has India any traditions for utilization of rest raw materials? What knowledge do you think the general public have about rest raw material, and that it can be used as a source to proteins and lipids?

4. Local fish markets generate a big amount of rest raw material. Is this utilized in any way or just discarded? In what ways could it be possible to make it profitable for the sellers to take care of this?

5. Rest raw material can be used to produce different valuable products, like feed, food ingredients, nutraceuticals and similar. Do you think there is a market for this in India? What laws and framework must be followed?

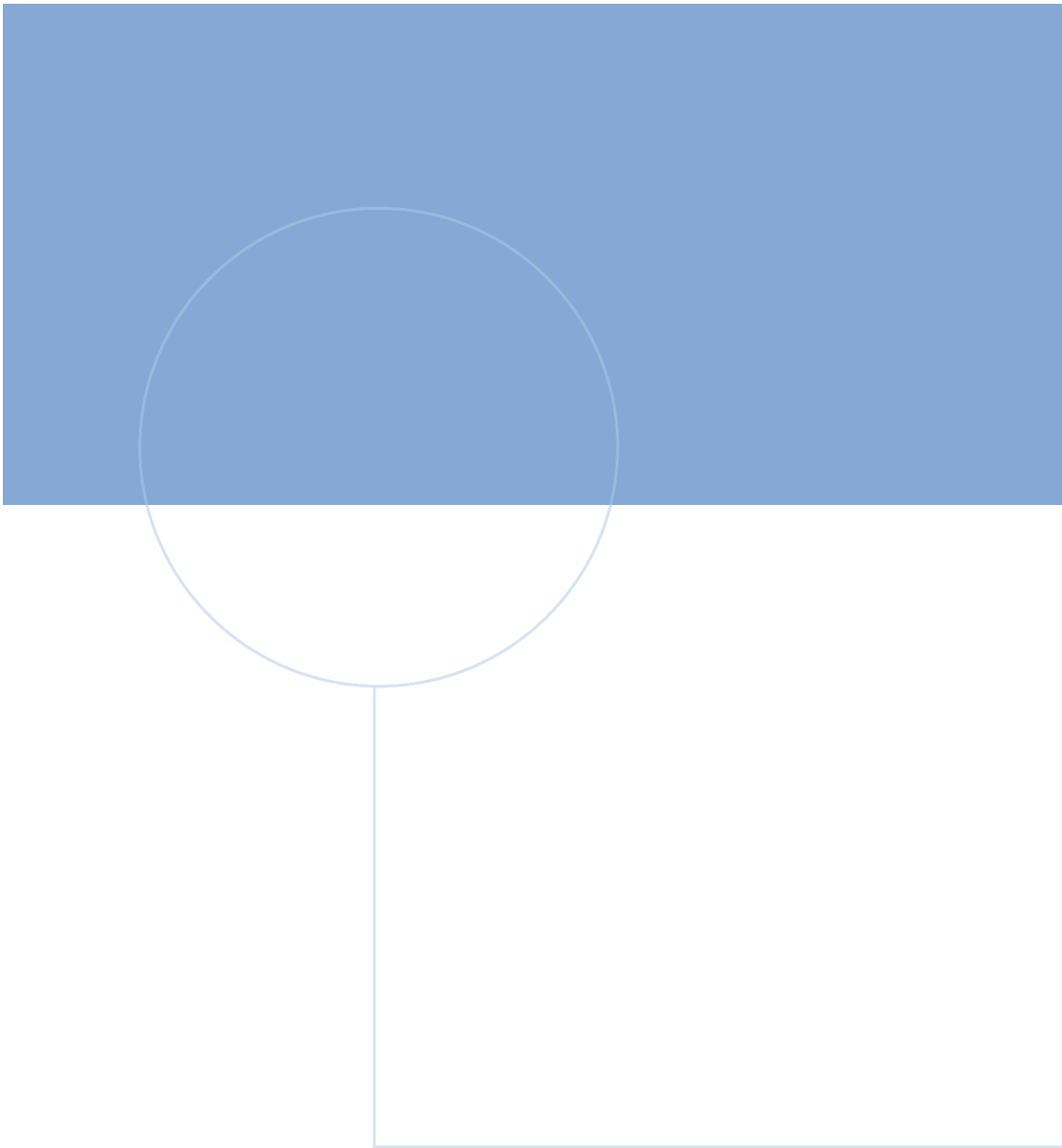
6. What are the major challenges in utilization of rest raw materials? Transportation, hygiene, inadequate technology, quality, consumer acceptance, others? Which aspects needs to be improved?

7. The UN has set several sustainable development goals they want to achieve by 2030. Goal 12 talks about responsible production and consumption. What needs to be done to achieve this goal?

8. Do you think value added products from marine rest raw materials would be accepted/consumed by vegetarians?

9. Regarding pollution, what are common threats found in Indian fish species? When utilizing rest raw material for human consumption these must be taken in consideration.

10. In what area does India benefit from a cooperation with Norway, and vice versa?



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