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New products from seafood side streams to stimulate a circular economy

Bachelor's thesis

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Gothenburg, Sweden 2019

Abstract

The Paris Agreement implicates that society will take necessary action to prevent the global mean temperature to rise more than 1.5°C. Since 80% of greenhouse gas emissions from agriculture originate from livestock, a protein shift is needed. This project has investigated the possibilities to implement proteins recovered from solid and liquid seafood processing side streams in different types of food products including fish ball, fish sausage, fish soup and mayonnaise. The proteins were recovered from fish processing by-products using the pH-shift method and shrimp processing water using flocculation. Effect of using the alternative proteins on proximate composition (protein, moisture, fat and mineral content), color, texture and sensorial properties of the products were evaluated. The fish soups were found as the least promising products, due to the low protein content and low scores in the sensory analysis. The results showed that 50% substitution of salmon protein isolate in fish balls and fish sausages with fish mince had no negative impact on the products quality but problems with a complete replacement may arise, especially regarding texture and color of the products. The mayonnaises containing both fish and shrimp proteins showed promising properties compared to the samples with egg protein. It was concluded that more studies of products with isolate are needed. Taste tests, different ratios, of mince and isolate, and repeats of already performed tests and products would provide useful future knowledge.

Sammanfattning

Enligt Parisavtalet måste samhället vidta nödvändiga åtgärder för att förhindra att den globala genomsnittliga temperaturen överstiger 1,5°C. Eftersom 80% av växthusgasutsläppen freån jordbruk kommer från boskap behövs ett skifte av proteinkälla. Det här projektet har undersökt om det är möjligt att implementera biprodukter som utvunnits från fiskrester och processvatten i fiskindustrin i olika typer av livsmedelsprodukter som exempelvis fiskbullar, fiskkorv, fisksoppa och majonnäs. Proteinerna från biprodukter från processering av fisk var framtagna genom användning av pH-skiftmetoden och för processvattnet från räkor användes flockulering. Effekterna på näringsinnehållet (protein, fukthalt, fett och mineraler) genom användningen av alternativa proteiner utvärderades på produkterna, även färg, konsistens och sensorisk analys utfärdades. Det visade sig att fisksoppan var minst lovande på grund av låg proteinhalt och låga poäng i den sensoriska analysen. Resultatet visade att ett utbyte med 50% av fiskfärs mot laxproteinisolat i fiskbullar och fiskkorv hade inga negativa effekter på produktens kvalitet men vid en fullständig ersättning av fiskkött mot proteinisolat kan problem uppstå, speciellt angående konsistens och färg. Majonnäserna som innehöll fisk och räkprotein visade lovande egenskaper jämfört med majonnäsen som innehöll äggprotein. Slutsatsen drogs att fler studier av produkter med isolat behöver göras. Smakprov, olika förhållanden av fiskfärs och isolat samt upprepningar av redan utförda tester skulle ge användbar kunskap i framtiden.

Contents

Abstract 2
Sammanfattning 3
1 Introduction 6
2 Aim 8
3 Theory 9
3.1 Protein shift and sustainable seafood production 9
3.2 Novel and alternative protein sources 10
3.3 Seafood Processing and generation of by-product 10
3.4 Protein and nutrient extraction from marine by-products 12
3.4.1 Seafood processing waters as a source of nutrients 12
3.4.2 Marine solid by-products 13
3.4.3 Protein isolate 13
3.4.4 pH-shift method 13
3.5 Seafood proteins and properties 15
3.6 Products on the market 16
4 Method 18
4.1 Planning of products 18
4.2 Material and chemicals 18
4.3 Development of products from fish and shrimp proteins 20
4.3.1 Fish soup preparation 20
4.3.2 Fish balls preparation 20
4.3.3 Mayonnaise preparation 21
4.3.4 Fish sausage preparation 21
4.4 Characterization of the products 21
4.4.1 Color analysis 21
4.4.2 Dry weight analysis 22
4.4.3 Ash content analysis 22
4.4.4 Texture analysis 22
4.4.5 Protein measurement using Dumas method 23
4.4.6 Sensory analysis 23
4.4.7 Method for extraction of total fat 24
4.4.8 Protein measurement using the Lowry method 24
4.5 Number of replicates and presentation of results 25
5 Results 26
5.1 Inventory template 26
5.2 Dumas method 28

5.3 Proximate composition of different products 28
5.4 Color measurement 30
5.5 Texture analysis 30
5.6 Sensory analysis 31
6 Discussion 33
7 Conclusions 35
8 References 36
Appendix A 40
Appendix B 56

1 Introduction

With a growing world population there is a higher demand for a sustainable and nutritious food supply. To prevent the global temperature from rising more than 1.5°C by the year of 2030. In line with the Paris Agreement, the livestock sector would have to reduce its greenhouse gas emissions by 49% (Harwatt, 2019). Therefore, we must find new sustainable alternatives to animal-based protein. There are a lot of novel and alternative protein sources in the market, but future foods are still quite underexplored (Tschirner *et al.*, 2017). However, a radical change in the food system from animal source foods to more sustainable foods needs to be implemented. The amount of novel and alternative food consumed on the market showed that consumers are interested in other types of food alternatives (Denny *et al.*, 2008).

Seafoods are broadly described as a source of high-quality protein, since it contains essential amino acids (EAA) and is easily digestible and absorbable (Tahergorabi *et al.*, 2011). Therefore, seafood is of great interest when looking for a replacement of protein-rich food products. Today there is a high awareness of the limited marine resources. In 2016 a capture of 90.9 million tonnes of fish was made in the world, a decrease compared to the previous two years (FAO, 2018). The total world fisheries and aquaculture accounts for 170.0 million tonnes, where 19.9 million tonnes were non-food uses. These facts speak for themselves; a more sustainable solution is needed. However, there is an increase in fish meals produced from by-products according to the state of world fisheries and aquaculture, this is a trend we need to continue developing. It goes hand in hand with what EAT-Lancet Commission says about how the world should act in order to feed the world population with healthy diets (Willett *et al.*, 2019). Two of the strategies described in the article mention halving the losses and waste of food, together with improving the management of the world's oceans. By-products, such as the remaining fish frame after filleting can be further processed into different products (Venugopal, 2006), these products can be *surimi*, sausages, powders and battered and breaded products.

Utilizing the by-products and extracting the protein is not without its complications though, as the by-products from marine processing are complex raw materials, with impurities such as bones and scales and high amounts of dark meat (Abdollahi *et al.*, 2019b). The dark meat has a high amount of heme protein (hemoglobin/myoglobin) and lipids (Curran Kenji *et al.*, 2009), potentially giving the product a brownish colour and rancid smell (Baron *et al.*, 2002). There are two promising methods for protein extraction that are in highlight for this project. One of the methods is based on extracting the protein from seafood processing waters and the other one is based on extracting the protein from the by-products, such as backbone and head, of seafood.

Large amount of water is used when filleting, transporting, storing and marinating of fish. Due to this fact, marine nutrients are leached into the water. One method for recovering nutrients such as proteins is called ultrafiltration-diafiltration (Amado *et al.*, 2016). Another method for extracting proteins from seafood processing waters is with chitosan complexes with polyanions such as alginate, Chi-Alg complex (Wibowo *et al.*, 2007). As stated earlier, there are several different processed waters. In the study (Osman *et al.*, 2015), four different herring industry processing water were analyzed, refrigerated sea water (RSW), processing water from cutting (PW), storage water (SW) and pre-salting brines (SB). The processed waters in the study were subjected to chemical characterization and biomolecule recovery electroflocculation (EF) and ultrafiltration (UF). From all the different processing waters for herring, pre-salting brines was shown to have highest protein and fatty acid content.

The process, in highlight, for recovery of proteins from solid by-product is the pH-shift method, developed by researchers at Massachusetts university (Hultin *et al.*, 2001) as an efficient process of isolating edible protein from animal products. In the process alkaline or acid solutions are used to solubilize the protein (Abdollahi *et al.*, 2018). The solubilized proteins are then separated from undissolved product with centrifugation or filtration and the wanted proteins are recovered with isoelectric precipitation.

The method is regarded as having high potential for extraction of proteins, used in food production, due to its high protein recovery yields, which has been shown for by-products of many different marine species (Surasani, 2018), and its ability to remove a high percentage of heme proteins and lipids (Abdollahi *et al.*, 2016; Kristinsson *et al.*, 2006). The extracted product has also been shown to have high amounts of EAA per gram protein and scores high on emulsification and foaming capacity, making it a good candidate for many different food products (Abdollahi *et al.*, 2018). Even though a lot of research has been done on the recovery of protein, from marine side streams, relatively few number of studies have been conducted on the potential applications of the recovered proteins in food products (Surasani, 2018) which has been targeted as main goal of the current project.

2 Aim

This project was carried out to contribute to reducing the waste that is generated during seafood processing and to find alternative protein candidates to meet the ongoing "protein shift". The main goal of this project was to see if it is possible to implement the recovered fish proteins from seafood processing side streams in different types of food products.

Specific objectives of the project were:

- To find suitable food product candidates for incorporation of marine protein enriched fractions by inventories at large food stores and web-based search.
- To develop new potential food products with the protein enriched fraction recovered from side streams of seafood processing.
- To evaluate proximate composition, color, sensorial and textural properties of the developed products.

3 Theory

3.1 Protein shift and sustainable seafood production

In order to meet the required goals for the Paris Agreement, all possible sectors in society will have to drastically reduce their emissions of greenhouse gas. The Paris Agreement implicates that society will take necessary actions to prevent the global mean temperature to rise more than 2°C this century, but also pursue efforts to limit the temperature rise even to 1.5°C (Masson-Delmotte *et al.*, 2018). Agriculture emits 24% of the total greenhouse gas and is the prime sector emitting methane and nitrous oxide (Harwatt, 2019). Close to 80% of the emissions from agriculture originate in livestock (Springmann *et al.*, 2016).

Suggested solutions to this problem can be several, but the solution proposed by Harwatt would be to gradually make a transition to a food source solely based on plants. However, one problem with entirely altering a diet from animal-based protein to plant-based protein is a question regarding malnutrition (Parodi *et al.*, 2018). Since aquatic and terrestrial animal protein is significant to reduce the malnutrition in low-income countries, altering completely to a plant-based diet could prevent the uptake of for example important macro- or micronutrients as stated by Parodi *et al*. One example is vitamin B12, which is found in for example meat, fish and dairy products. An appreciable level of vitamin B12 will not be able to obtain with an entirely vegan diet. This deficiency can lead to bone marrow failure and nervous system disease (Stabler, 2013). However, according to Parodi *et al.*, consumption of red meat can lead to cancer or coronary heart diseases. Hence, this is yet another reason to replace these types of foods from human diets. As described by Springmann *et al.*, the death rate could decrease significantly, if shifting to a plant-based diet. This number would lie between 6 and 10% on a global level.

But what if we would not entirely shift to a plant-based diet, but focus on aquatic proteins instead? As fishes are source of for example vitamin D and essential minerals as calcium, zinc and magnesium, it makes a healthy source of protein (Mohanty *et al.*, 2017). It is also stated that fish is an important food to prevent malnutrition in developing countries since certain fish species can have a relatively low price.

Aquatic animal protein is a sustainable alternative to plant-based foods, if it is produced in an environment-friendly way (Jordbruksverket, 2012). Overexploitation of fisheries is demanding new sustainable solutions to reassure our ability to produce fish (Godfray *et al.*, 2010). One way would be to expand the aquaculture, which is important for the global production of not only fish but also crustaceans and other types of aquatic organisms (Jordbruksverket, 2012). Aquaculture is already resulting in more fish and crustaceans than conventional capture fisheries (Tacon *et al.*, 2015). It is stated that from 2000 to 2012 the output from aquaculture has more than doubled, starting at 41.7 million tonnes and resulting in 90.4 million tonnes the year of 2012. But one problem is that more than 70% of the global aquaculture is reliant on external inputs from capture fisheries as feed. As stated by Godfray *et al.*, other complications when it comes to aquaculture are for example chemicals, that are used for treating the fish, that later are leached out into lakes, seas and other water bodies. If the fish from aquaculture would manage to escape into the wild, this could also risk spreading disease and genetic contamination. Aquaculture could also lead to eutrophication since it emits phosphorus and nitrogen (Albertsson *et al.*, 2012).

Godfray *et al.* stated that improvements of aquaculture in the coming future could be to use waste from agriculture as nutrients. But improvements can also be done by putting more effort in finding the most promising and suitable locations for fish farming, better stock selection and selecting the most preferable fish species. Albertsson *et al.* wrote that a solution to the eutrophication problem could be to farm more oysters and blue mussels, since they consume plankton and algae. Treating the fish as decent and humanely as possible and working preventively, decrease the risk of diseases and will be required as well (Jordbruksverket, 2012).

A field that there has not been as much focus on, is the one regarding so called future foods. Future foods can be for example cultured meet, seaweed and different types of insects (Parodi *et al.*, 2018). A diverse diet of future foods could be an alternative to find edible substances with for example the right micro- and micronutrition's. Parodi *et al.* also stated that levels of iron, zinc and calcium are for example as high in certain types of insects than in conventional beef.

3.2 Novel and alternative protein sources

Sustainable food system includes a shift towards plant-based dietary patterns, such as soybeans and different crops (Willett *et al.*, 2019). The focus has been on the consumption of fruits, vegetables, legumes and nuts must increase while consumption of less healthy food such as animal-source foods need to decrease (Lang *et al.*; Parodi *et al.*, 2018). The focus on consumption has left the potential of future foods fairly underexplored, future food such as insects, seaweed, chlorella, spirulina and cultured meat. Containing all essential micronutrients and a relative high amount of protein, makes future food a good alternative for animal-source foods compared to plant-source foods (Tschirner *et al.*, 2017).

Seaweed, also called macroalgae, is a nutrient-rich food for human consumption and has the capacity to grow in massive amounts without chemical fertilizer, land or access to fresh water (Tiwari *et al.*, 2015). The seaweed industry has over the last decade increased with an exponential growth and 2012 farmed seaweed reached 24 million tons. Seaweed is protein rich and some species even contain more protein than conventional protein-rich foods (Rajauria *et al.*, 2015). Benefits of seaweed are that algae can easily grow in marine, fresh and wastewater environments, creation of new fish habitants and responsibility of 50% of the global carbon fixation and oxygen production.

Microalgae is also known for being protein rich (50-60%) and are usually used as a whole-food ingredient which are favorable compared to soy proteins where the protein is isolated from the plant (Shelef *et al.*, 1984). Chlorella and Spirulina are the most used microalgae, but other species are gaining more acceptance with time.

Quorn is the trademark for mycoprotein and consists of high-protein, high-fiber and low-fat content and have therefore been acknowledged for its health benefits aspects (Denny *et al.*, 2008). The negative aspect of mycoprotein is the high energy consuming fermentation process to maintain a constant temperature, the heat treatments and the use of centrifugation (Parodi *et al.*, 2018).

Insects have a high potential of becoming a well-established future food. In vitro models have shown that bioavailability of micronutrients in insects is comparable to those in beef (Parodi *et al.*, 2018). According to Parodi, the protein digestibility in seaweed ranges from 56-90%, however, compared to milk casein; mycoprotein was found to be 15% lower, spirulina 25% lower and chlorella 30% lower in sense of protein digestibility. It is important to have knowledge of novel and alternative protein sources to be able to produce quality seafood products of by-products.

3.3 Seafood Processing and generation of by-product

With the increasing demand on ready ingredient; foods are more and more processed into easy-to-use ingredient which require less cutting or cleaning before cooking, for instance sliced vegetables, sliced-pre-cooked potato, filleted fish, peeled shrimp and so on. These types of processing produce lots of by-product which would not be generated or would be generated in less quantity providing the consumer would rather use raw food. Seafood processing is generating huge amount of solid by-product as i.e. only 30-40% of a fish is turned into fillet where 60-70% is being lost from food chain as solid by-product, which still consists high amount of valuable nutrients (FAO, 2018). In 2016, 170.9 million tonnes (MT) of seafood were captured by fisheries or produced by aquaculture. About 151.2 MT were used for direct human consumption. In Europe, 5.2 MT fish is captured, and 1.3 MT are produced by aquaculture (Frid *et al.*, 2012). 3.8 MT of the catch is used for human consumption and the rest for industrial processes such as fishmeal and oil production. Waste from fish processing at sea is generated by capture fisheries where approximately 8%, which corresponds to 410 000 tones in Europe, can be discarded back into the sea.

A high amount of nutrition is lost in seafood process wastewater. In Sweden, protein loss during marination of herring both in primary and secondary producers amount to 4,5 and 20% of ingoing protein, respectively, the losses are equal to 23 and 82 tonnes of protein for processing of 3500 ton of herring annually in both processing companies (Forghani *et al*. unpublished data). Another example is from shrimp peeling processing where protein loss is 10% of ingoing protein thus amount to 8 ton of protein during processing of 900 tonnes of shrimp annually.

Based on official data, the total amount of whole fish from imports of fish, landings and aquaculture are 609 000 tonnes where 115 000 tonnes of whole fish were available for processing (Bergman, 2015). Furthermore, 63 000 tonnes of co-products were generated in processing. The 63 000 tonnes include human consumption in Sweden as well as exported human consumption. Most common fish species are whitefish, salmon and pelagic fish. Based on interviews with different processors in Sweden, around 30 000 tonnes of seafood co-products are generated each year. Bergman reported that co-products are mostly used for feed for mink, fish meal and oil for aquaculture and other animal feed. The main reason why the processors do not focus on human consumption is because it is not seen as profitable. People in Sweden is less likely to eat the head of the fish for example. Moreover, it requires more time, more working staff as well as more careful handling and processing than production of fish meal.

After filleting the fish, more fish meat can be isolated from the remaining fish frame by using mince separation techniques (Venugopal, 2006). By-products remaining after filleting of salmon, include heads, backbone, trimmings containing muscle, skin and bone as well as viscera (Benjakul *et al.*, 2019). Benjakul *et al.* further described that from these leftovers, collagen, gelatin, oils and hydrolysates can be derived which can be incorporated in products. Venugopal reported that with fish mince a diverse range of products can be developed such as *surimi*-based seafood analogs, sausages, dried fish flesh flakes, powders and frozen battered and breaded products. Meat can be separated from many different fish species. One common method to isolate the fish meat is by using a type of machine called the meat-bone separator or deboner. Headed and gutted fish will pass between a perforated drum and a rotating belt where the fish skin and bone stay outside the drum and scraped off while muscle and fat will go through the drum. Venugopal described that the quality of the mince, with respect to the content of scales and bones depends on the dimensions of the orifice of the drum. Normally, the orifice size is between 3 to 5 mm. A higher disintegration of the mince will be generated by smaller orifice size (Benjakul *et al.*, 2019) .

By-products can be divided into two large groups, one with easily degradable by-products such as fractions of viscera and blood (Rustad *et al.*, 2011). The second group is relatively stable by-products such as bones, head, frame and skin. Rustad *et al* described that the two groups must be treated separately with special care in order to reduce microbial spoilage. The quality of the fish mince and by-product generated upon processing fish depends on handling, how it is processed and the harvest season (Venugopal, 2006).

Many of the products and the processes mentioned have lack of desired progress in utilization (Venugopal, 2006). Venugopal described that the problem is that the technical innovations have been technology-driven rather than market-driven on process and product levels. Therefore, consumer acceptability is important and great market strategies must be developed. Consumer acceptability includes design of appealing products, preferences varying in cultural backgrounds, quality standards and careful process development. One way towards better consumer acceptability is to incorporate extracted proteins from fish by-products into products already existing on the market.

3.4 Protein and nutrient extraction from marine by-products

3.4.1 Seafood processing waters as a source of nutrients

Huge quantity of water is used for filleting, transportation, storage and marination. While the water is used, marine nutrients are leached into these waters. Nutrients such as protein and astaxanthin can be recovered with ultrafiltration-diafiltration (Amado *et al.*, 2016). The study showed that UF of shrimp cooking wastewaters with 300, 100, 30 and 100→30 kDa Molecular Weight Cut Off membranes is an effective method of concentrating the protein in waste waters. Concentrations of 10-13µg/L Astaxanthin can also be recovered after UF at 300 kDA. Oil from northern shrimp, which is predominated by triacylglycerols and astaxanthin esters, is rich in omega-3 fatty acids, can also be recovered from Northern shrimp cooking waters (Jiao *et al.*, 2015). Jiao presented the characterization of triacylglycerols and astaxanthin esters by using HPLC-HRMS, MS/MS and C-NMR, in combination with FAME analysis. Jiao also said that the characterization of the components in the Northern shrimp oil which is recovered from shrimp cooking waters, provides an understanding of the healthful benefits of the oil as a supplement or food ingredient. In another study (Gringer *et al.*, 2015), the low molecular weight compounds such as proteins and phenolic compounds, which are present in salt brines from marinated herring production, showed to be a good source of antioxidants.

There are several different processing waters for herring such as, refrigerated sea water (RSW), processing water from cutting (PW), storage water (SW) and pre-salting brines (SB). These herring industry processing waters were exposed to chemical characterization and biomolecule recovery using electroflocculation (EF) and ultrafiltration (UF) (Osman *et al.*, 2015). Osman wrote that EF separates organic molecule without the addition of chemicals while UF is used as a main concentration technique. The study showed that highest protein and fatty acid content were found in pre-salting brines, up to 12.7 ± 0.3 and 2.5 ± 0.1 g /L, respectively. While the lowest protein and fatty acid content were found in Refrigerated sea water. Osman also wrote that each ton of final herring product requires up to 7 m^3 water. Another way to recover protein from processed water is described in (Wibowo *et al.*, 2007). Wibowo showed that soluble protein recovery by chitosan (Chi) complexes with polyanions such as alginate (Alg) is more effective that using chitosan alone. The concentration of the Chi-Alg complex that were used were 20, 40 and 100mg/L surimi wash water (SWW). The study showed that at the lowest concentration tested, 20 mg/L SWW, the experimental chitosan SY-1000 gave higher protein recovery that a commercial sample (CHI-84) used in previous studies. For the CHI-84 to be as effective in protein recovery as SY-1000, the concentration needed to be a 5-fold higher. Wibowo also wrote that if the Chi-Alg were to be implemented commercially, it would not only be a more effective alternative in recovering soluble proteins in processed waters but also a better choice due to costs.

In order to produce a good food product with proteins from marine processed waters, the protein compound does not only need to be of high value, it also has to have a desirable taste and smell. Enzymatic hydrolysis, thermal reaction, lipid oxidation, and environmental and microbial pollutions are all reactions that are involved in the creating of the shrimp aroma (Céline Jarrault *et al.*, 2017). In the study, a natural flavoring concentrate from shrimp cooking juice was produced by three different processes; nanofiltration (NF), volume reduction ratio (VRR) of 10, and osmotic evaporation (OE). In NF, shrimp cooking juice is nanofiltrated for production of water with lower chemical oxygen demand and for concentration of high valuable substances. OE is the process where the flavoring concentrate is separated from the brine on a dry matter basis. The conclusion of this study showed that NF modified the smell of the concentrate somewhat, in a positive way, while the OE proved to be successful in the process of separating the flavoring concentration from the brine with up to 52% dry matter.

3.4.2 Marine solid by-products

Many of the marine by-products that have potential to be sources for high value food-grade protein products, such as head, backbone, tail and trimmings, have a high amount of dark meat as well as a complex bony structure (Abdollahi *et al.*, 2019b). The dark meat of a fish, found directly under the skin, has higher amounts of hemoglobin/myoglobin (Hb/Mb) and lipids (Curran Kenji *et al.*, 2009). The presence of heme-pigments (Hb, Mb) can have effects on derived protein products' qualitative parameters, like whiteness, microbial growth and lipid oxidation (Abdollahi *et al.*, 2016). Hb/Mb in oxidized forms have shown to contribute to oxidization of lipids, through several mechanisms, giving the product a greyish-brown colour and a rancid smell (Baron *et al.*, 2002).

The complex raw materials, in the by-products, have presented challenges in removal of lipids and heme proteins as well as other impurities (e.g. bones, scales and connective tissues) while still recovering protein with retained functionality (Abdollahi *et al.*, 2016). These challenges have led to continuous research on methods of protein recovery.

3.4.3 Protein isolate

Protein isolates are a group of products where raw material has been refined to have a high protein content. There are several different types of protein isolates; soy protein isolate is refined from soy beans, whey protein isolate is a widely used protein powder where the protein in left over whey, from cheese manufacturing, is concentrated by centrifugation, vacuum evaporation and spray drying (Etzel, 2004). Fish protein isolate (FPI) is a type of protein isolate, where fish protein has been purified so that the protein content accounts for at least 90% of the products dry weight (Rustad *et al.*, 2011). Rustad wrote that the term FPI is mostly used for protein powder from marine raw material that has been processed with the pH-shift method. There are also other levels of purity, e.g. concentrated protein powder, where the protein content account for a lower percentage of the dry weight.

3.4.4 pH-shift method

The pH-shift process was developed by researchers at Massachusetts university (Hultin *et al.*, 2001) as an efficient way of isolating edible protein from animal products using alkaline or acidic solutions to solubilize the protein. Abdollahi described the method as a process that involves a selective extraction of proteins, using shifts in pH (Abdollahi *et al.*, 2018). The pH-shifts will increase the positive or negative charge of the protein side chains, in acidic and alkaline solutions respectively; increasing repulsion between protein molecules as well as increasing interaction between the protein side chains and water, causing the solubilization (Surasani, 2018). The proteins of interest are then recovered by isoelectric precipitation and water is removed by filtration or centrifugation. The protein isolate can then either be directly frozen, after being mixed with cryoprotectants, or dried into a fish protein powder (FPP) and be used for development of wide range value added products. The method has been successfully tested in lab and pilot scale for protein recovery from a wide range of raw materials, such as whole fish, fish by-product, poultry by-product, krill, mussel and seaweed (Abdollahi *et al.*, 2019a; Gehring *et al.*, 2011; Shaviklo, 2008).

An important parameter for the economic potential of the pH-shift method, is the yield of the proteins of interest (Surasani, 2018). Surasani wrote that several studies have been conducted on the pH-shift process and that the process has been found to have higher yields of recovered protein, compared to the conventional, three cycle, washing process for surimi. The most important factor that affects yield rates is the solubilization at alkaline and acidic pH. Significant differences, for protein recovery rates, have been shown between by-product from different species and using an alkaline or acidic process ranging from 31 to 98% depending the type of raw material, process version and yield measurement method. Continuous research at Chalmers University of Technology, led by Undeland, has worked to refine and evaluate the method. The group has produced several reports on improvements of the method and evaluation of the products. They concluded that an alkaline pH-shift process could remove considerably more Hb than an acidic process, in cod mince (Abdollahi *et al.*, 2016). If the precipitation of the proteins was made at pH 6.5 instead of pH 5.5, the heme removal was increased further, up to 91% in the alkaline process.

The pH-shift process has both advantages and limitations compared to other processes for protein recover, like the surimi process. The high rates of removal of heme proteins and lipids are major advantages (Kristinsson *et al.*, 2006). Lipid oxidation can give the product a rancid smell. Kristinsson *et al* have shown lipid reductions of 68.4%, when the alkali process was used on Atlantic croaker, compared to only 16.7% in the conventional surimi process. Disadvantages of the process are low protein recovery rates and unwanted color of the product, for some species. Nolsøe *et al*. found that replacing centrifugation with filtration improved protein yield and color for isolate from blue whitling, but also increased the amount of lipids in the product (Nolsøe, 2011).

Abdollahi and Undeland describe the nutritional, structural, functional and sensorial properties of FPP (Abdollahi *et al.*, 2018) that has been procured, through the pH-shift process (Hultin *et al.*, 2001), from by-products of three different fish; salmon, cod and herring.

The authors concluded that the amount of EAA per gram protein were higher for FPP derived from each of the three fish origins compared to the corresponding raw materials and soy protein isolate. Differences in sensorial and functional properties between the different FPPs were found. Emulsion and foaming capacities were as good for the FPPs as for egg white protein powder (EWP) and soy protein isolate at high pH. The emulsion activity indexes (EAI) for the FPPs were lower than for EWP at pH 5-7 but the emulsion stability index (ESI) was higher, for all FPPs compared to EWP, at pH 9-11. The FPP derived from cod by-products showed better emulsion and foaming capacity than the protein powders from salmon and herring. Colour readings showed that FPP from cod and salmon had a high whiteness while herring protein powder scored lower due to its dark brown colour. The sensorial properties, judged by six skilled panellists, showed the best result for cod protein powder while herring protein powder showed higher levels of fish and lipid oxidation-related flavour and odour. The researchers conclude that the FPPs have potential for use as food ingredients but the differences in properties will determine their potential applications.

Surasani concluded that many reports have been published on the recovery of protein from marine by-products, but few studies have looked at the potential food product applications of the isolate (Surasani, 2018). Nolsøe and Undeland concluded that value adding of underutilized muscle proteins is the area where the pH-shift process has the largest potential (Nolsøe *et al.*, 2009). Shaviklo prepared fish balls using proteins from a pH-shift process of haddock and found that the cook loss was similar to pure mince balls and concluded that the recovered protein could be used to develop mince-based products (Shaviklo, 2008). Ibrahim also prepared fish balls using isolates from small Nile bolti fish and concluded that the use of isolate improved the protein content of the product but reduced the fat and carbohydrate content (Ibrahim, 2015). Isolates, procured from the process and dried into powder, could be used as a batter to create low-fat fried products (Nolsøe *et al.*, 2009). Further studies are needed of the potential applications of the recovered protein, if the pH-shift process is going to have an impact on the recovery and use of marine by-products.

3.5 Seafood proteins and properties

Marine sources, in other words fish, shellfish, mussels and more, are a source of food that can contribute to humans in many ways (Venugopal, 2008). Marine sources are an excellent source for both functionally active and nutritive proteins and have always been considered as a relatively cheap animal source. Proteins are essential for humans in the way of growth and body maintenance as providing amino acids.

Venugopal discussed that the protein content of raw fish muscle varies much based on species. In raw finfish the average protein content is 19% and red meat and fish are comparable in the sense of amino acid pattern, but stroma proteins (collagen, elastin and gelatin) are only 3% in fish meat compared to red meat (Venugopal, 2008).

Tahergorabi *et al* stated that the part used to derive different food products is the muscle tissue (Tahergorabi *et al.*, 2011). It can be divided into two groups; striated and smooth, where the striated muscle consists of two groups; white and dark meat. The white meat can be found in every part of seafood, while the dark meat is present underneath the skin. The most important part of the muscle tissue is the proteins. More precisely 15-25% of the total weight consists of proteins according to Tahergorabi *et al*. There are three major groups of proteins. The first one is the myofibrillar proteins, they account for 66-77% of the proteins in fish muscle. The myofibrillar proteins consist of myosin, the thick filament, and actin, the thin filament (Curran Kenji *et al.*, 2009). The quality of the myofibrillar proteins decides the texture development, which is of importance for the consumer.

The sarcoplasmic proteins are the second major group, they are water-soluble. During gel matrix formation they hinder myosin cross-linking, because of their insufficient water-holding capacity and because they do not gel, according to Tahergorabi *et al*. The last group is the stroma proteins, which are water insoluble. In dark fish meat they are more common than the sarcoplasmic proteins (Curran Kenji *et al.*, 2009). Proteins from marine sources have some major functional properties in foods, for example solubility, gelation, emulsification and whippability and foam stability.

One of the most important physiochemical properties when considering the production of muscle food products is the solubility of the protein (Tahergorabi *et al.*, 2011). Proteins in muscle food have a significant amount, 75%, of water bound in different basic forms (Venugopal, 2008). The solubility is a determination of the extent of these interactions.

During cooking and comminution, fish muscle proteins hold water molecules, this ability is called water-holding capacity (WHC). In food processing, water binding compounds are often added to increase the WHC of the product, affecting the sensory attributes of the product i.e. how succulent and juicy it's perceived (Curran Kenji *et al.*, 2009). Protein isolate from fish by-products could be used as such a binding compound, increasing the perceived quality of the food product.

Protein gelation is often referred to as the transformation of protein from a sol to a gel-like state, and a gel is referred to as the intermediate between solid and liquid state (Tahergorabi *et al.*, 2011). A gel has cross-linked strands of chains of either proteins or carbohydrates (Tahergorabi *et al.*, 2011; Venugopal, 2008). The chains form a three-dimensional network. By incorporating ingredients such as salt, polyphosphate, starch and other proteins from another source, a change in the rigidity of the gel can be obtained according to Venugopal.

Emulsion is a mixture of two phases that usually do not mix with each other (Tahergorabi *et al.*, 2011). Emulsification is when a reduction of this interfacial tension between the two phases occur. There are three types of food emulsions; oil-in-water or water-in-oil, foam and sol (Venugopal, 2008). An example of a good emulsifier for food products and oil, according to Tahergorabi *et al*, is fish muscle proteins, since they contain hydrophobic amino acids.

Protein-stabilized foams are shaped while shaking a solution (Tahergorabi *et al.*, 2011). Tahergorabi *et al* referred to a foaming property as "its ability to form a thin tenacious film at gasliquid interface so that large quantities of gas bubbles can be incorporated and stabilized". The main surface-active agents who stabilize the gaseous dispersed phase in food products are proteins (Venugopal, 2008). Tahergorabi *et al* discussed the best foaming properties, which belong to fish protein concentrate with amphiphilic characteristics. It is important to understand the importance of the protein properties when producing new products.

3.6 Products on the market

A lot of fish proteins are made into value-added products, but it is important to remember that the final product will not have a higher quality than the raw material (Peterson, 2010). That is, as good as the finfish or shellfish. It is important to have information about the fish or shellfish the product comes from to be able to draw conclusions about the products. Breaded seafoods from fish fillets, portions or shrimp are the most common. Often products are raw and frozen when they are sold. Some products common in the food market are fish sticks, nuggets, patties, melts and stripes. There are also a lot of meat analogues in the food market such as burgers, hams, hot dogs and sausages.

Today side streams have been used to produce seafood protein powders, seafood protein hydrolysates, food coating films and fish meal (Tahergorabi *et al.*, 2011). Seafood protein powders can be produced by either grinding, heating, sieving, centrifugation and dehydration or by pH extraction and isoelectric precipitation. Today the seafood protein powders are used as animal feed ingredients and in dietary supplement writes. Protein hydrolysates are proteins from fish muscle that are subjected to hydrolysis where proteins are enzymatically or chemically broken down into smaller peptides (Benjakul *et al.*, 2019). Protein hydrolases can be used in different ways in the food industry such as protein supplements, milk replacers, stabilizers in beverages as well as flavor enhancers in confectionery products (Kim, 2014). Other food systems that fish protein hydrolysates have been successfully incorporated in are fish and meat products, cereal products, crackers and desserts (Chalamaiah *et al.*, 2012).

Another example is to modify the seafood proteins into a bio-degradable super-absorbent hydrogel. This could potentially replace non-biodegradable hydrocarbon-based hydrogels, used for instance in diapers and paper towels (Benjakul *et al.*, 2019) (Curran Kenji *et al.*, 2009). Fish by-products not suitable for human consumption can be used as animal feed. It is the European Food Safety Authority that decides whether fish by-products are safe for animal feed (Hayes *et al.*, 2019). Fish food coating films that are derived from proteins, polysaccharides and lipids. The food coating films are of interest since they can be used as a natural resource in the packaging industry, and they can improve the stability of frozen fish products (Tahergorabi *et al.*, 2011). Fish meal is used in animal feed, it is possible due to the high nutritional value. Fish silage is also produced from underutilized low-value fish and side streams. It is used in animal feed as well, as a protein supplement.

A conventional product from fish mince is *Surimi* with origins from Japan, which is washed and preserved fish meat with a great capacity to form gels (Venugopal, 2006). It can be used to produce many products such as imitative lobster tail, shrimp and crab legs as well as sausages and fish balls. *Surimi* is both made of fish fillets but also fish frames from filleting operations. It is possible to recover up to 60% of meat from the fish frames. Production of *surimi* requires many washing steps to remove certain components that impair frozen storage capacity (Rustad *et al.*, 2011). The washing steps generates a high amount of process wastewater.

4 Method

The flow chart of the project can be seen in Figure 1.

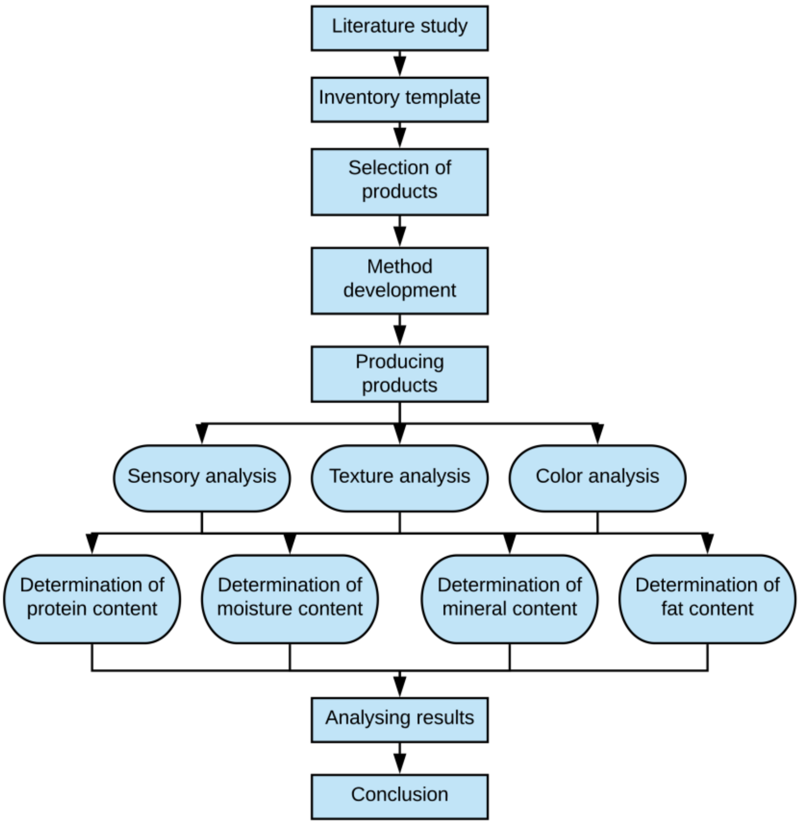

Figure 1 – Flow chart of the project.

4.1 Planning of products

The first step of deciding which products to produce was to visit a supermarket. The products that seemed interesting were put into an inventory template, as can be seen in Figure 1. In the template, for example the protein content, functionality and potential for application were noted, see Table 2. After a meeting with the supervisors the most promising products were chosen. All the products in the inventory template were not produced, this was due to the time schedule and to get a variation of the products produced.

4.2 Material and chemicals

Sodium oxide (NaOH), hydrochloric acid (HCl), sucrose, tripolyphosphate, chloroform, methanol, copper sulfate pentahydrate ($CuSO_4$), sodium carbonate (Na_2CO_3), sodium potassium tatarate (Na-k-tatarate), sodium dodecyl sulfate (SDS), phenol, folin-ciocalteu phenol, shrimp protein powder (ShPP), salmon protein powder (SaPP), cod protein powder (CPP), egg white protein powder (EWP), salmon mince (SMB) and salmon protein isolate (SPI) were provided by Department of Food Science at Chalmers University of Technology. SMB was produced by meat-bone separation from fresh salmon backbone and here it was used as a control to imitate what was used in the industry. SPI and CPP were produced from fish head and backbone with alkali-aid and pH-shift processing. SMB and SPI can be seen in Figure 2. The protein powders were produced by freeze-drying the mentioned protein isolates. The ShPP was made by flocculating shrimp boiling water using alginate and subsequently it was spray dried. The EWP was a commercial protein.

In Table 1 protein, dry matter, moisture, ash, fat and carbohydrate content can be seen for the different proteins used.

Table 1 – Composition of the different proteins.

Dried biomass	ShPP	SaPP	CPP	Salmon by-product	SMB	SPI
Protein (N analyser) (%)	61.7±0.1	67.74± 5.82	84.55± 2.92	34.60 ± 0.93	46.78 ± 1.5	72.33 ± 0.52
Dry matter (%)	92.2±0.03	91.7	92.7	-	-	-
Moisture (%)	7.7	8.3	7.3	57.95 ± 0.11	68.02 ± 2.43	80.69 ± 0.00
Ash (%)	2.7±0.0	2.44±0.04	84.55±2.92	28.38 ± 0.45	3.70 ± 0.53	2.18 ± 0.04
Fat content (%)	26.7±1.2	32.06±0.97	10.14± 0.28	52.03 ± 2.96	43.11 ± 1.32	20.58 ± 0.24
Carbohydrate (%)	1.5	-	-	-	-	-

Figure 2 – Representative picture of SMB (left) and SPI (right).

4.3 Development of products from fish and shrimp proteins

4.3.1 Fish soup preparation

The fish soup recipe was inspired by Kelda archipelago soup from Arla Foods (Arla, 2019). See Appendix A, Table 9 and 10 for raw data. The first step for the fish soup was to chop the onion and the fennel. A half tablespoon of oil was used for the frying. Then 125 g of water, 30 g of white wine and 3.5 g of ShPP were added to the pan. The soup then boiled for approximately 10 min under a lid. Afterward, 42 g of 1.5% milk and 25 g of 27% cream were added to the soup along with 3.3 g of the potato starch. The soup boiled for 5 min and then the soup was mixed to a creamy consistence, see Figure 3. The soup was distributed to different tubes and then marked. The same procedure was repeated for the second soup, but the ShPP was switched out for 2.68 g of SaPP, see Table 1 for composition of the powders.

Figure 3 – Homogenity for fish soup, with ShPP, before mixing.

4.3.2 Fish balls preparation

The cooking method for fish balls was prepared following (Chen *et al.*, 2011). See Appendix A, Table 14-16 for raw data. One prototype of fish ball as a control was prepared with 99.8 g SMB, see Table 1 for composition. The SMB was chopped in a mixer together with 7.5 ml milk, 6 g potato flour and 2.8 g salt during 1.5 min. Then the weight of the mixture was measured, divided into 4 equal parts and manually formed into balls, see Figure 4. The weights of the balls were also measured.

Then the fish balls were subjected to a precooking at 45°C for 15 min followed by cooking 87°C for 2 min. The samples were then cooled down by soaking in cold water (0°C) for 5 min. Finally, the fish balls were kept in room temperature for 30 min and thereafter the weights were measured again. The weights of the fish balls were ranging between 18.5-23.4 g.

The same cooking method was used in order to make a prototype with 50% SMB and 50% SPI or 100% SPI. The ratio of other ingredients was similar to what was used in the control fish ball. The only difference is that the SMB and the SPI were mixed alone for 25 sec before the rest of the ingredients were added and mixed for 1.5 min. Moreover, three balls were formed. The weights of the fish balls were ranging between 18.6-21.9 g.

In order to analyze the moisture, fat content, protein and ash, 1-2 g of each prototype were placed as triplicates in small plastic bags and stored in a freezer for later use. A color analysis of the fish balls was performed. The fish balls of each prototype were split in half and placed in an empty clear Petri dish plate and analyzed by the Konica Minolta, Cr-400 equipment.

Figure 4 – Representative picture of dough texture for fish balls of 100% SMB (left) and 100% SPI (right).

4.3.3 Mayonnaise preparation

Mayonnaise is an oil-in-water emulsion and it was prepared following the method explained by Abdollahi & Undeland (2018), using CPP, ShPP and EWP (as control). See Appendix A, Table 4 and 5 for raw data. The first step was to weigh distilled water in a beaker and then the protein for each sample were weighed on the same scale. The protein sample (3 g) was dispersed with 32 g of distilled water with a magnetic stirrer for 30 min.

The pH of the protein dispersions was measured with a PHM210 standard pH meter, MeterLab. First it was calibrated with pH 4 and pH 7. The shrimp sample was below pH 7, and it should be 7 (see Appendix B, Table 24) therefore it was adjusted with 1N and 0.1N NaOH. The pH of EWP sample was above 7 and it was adjusted with 1N and 0.1N HCl, to pH 7. 0.2 ml of lemon was added to all samples for the flavour, and then the pH was measured again, before and after the lemon was added. Then, 65 g of sunflower oil was weighed and added to each of the samples. An IKA T18 digital ULTRA TURRAX homogenizer was used to conduct the emulsification at 15.000 rpm for 2 min.

4.3.4 Fish sausage preparation

Emulsion fish sausage prototypes containing 40% plant oil was prepared following the method explained by (Panpipat et al., 2008) with some modifications. Firstly, a control of fish sausage with 100% of SMB (Table 1) was made. Later, a prototype of fish sausage with 50% SMB and 50% SPI was made.

For the control, 139.7 g of SMB was prepared and put in the chopper for 40 sec to mix and become a batter. Then, 3.9874 g of salt was added to the batter. The batter was then further chopped for 2 min. After the second chop, 40 g of vegetable oil was added to the batter and put to chop for 2 more min. Lastly, 3.5162 g of potato starch, 3.0525 g of sucrose, and 1.4930 g of tripolyphosphate were added to the batter and chopping continued for 3 more min, with a pause of 10 sec between each minute to gather all the batter on the wall of the chopper and put it in the middle where the chopping occurs. After the last ingredients were added to the batter and then chopped, an eye control of the batter occurred in order to see if the batter was smoothly chopped. The batter was then stuffed, with a stuffer and funnel, into the plastic sausage casing. The batter was compressed tightly in the casing before the knots at each end were tightly tied.

Fish sausage containing salmon protein isolate was also prepared by replacing 50% of SMB with SPI and all other ingredients and preparation was similar to what is mentioned above.

The method for the prototype had similar steps as the method for the control. The only two things that differed were the proportion of the different ingredients and the first chopping time which was 40 sec for the control and 1 min for the prototype.

Both the control and the prototype were later incubated at 40°C for 30 min before the two products were cooked at 80°C for 15 min. The products were put in cold water with ice for 15 min. See appendix A, Table 6 and 7 for raw data.

4.4 Characterization of the products

4.4.1 Color analysis

Color analysis was done on all the prepared products according to the method (Abdollahi *et al.*, 2018). One slice of the different products each were placed on an empty and clean petri dish. The plates were then placed on the chroma meter of the brand Konica Minolta, Cr-400. The data given in the analysis was three light parameters on all the products, see Appendix B, Table 21 for raw data. The parameters on each sample from all the products were later analysed.

The calculation for whiteness was done following Equation 1 for all the samples,

$$Whiteness = ((100 - L*)^2 + (a*)^2 + (b*)^2)^{1/2} \quad (1)$$

while redness was calculated with Equation 2.

$$Redness = \frac{a*}{b*} \quad (2)$$

4.4.2 Dry weight analysis

Firstly, the scale was reset with the container and then 2-3 g of each sample were added for each container, see Appendix B, Table 31 for raw data. The container was then placed in an oven overnight, the temperature for the oven was 105-110ºC. Next day the samples were weighed again.

The dry weight was calculated using Equation 3, 4 and 5.

$$(Water\ content\ [\%]) = \frac{(Water\ [g])}{(Wet\ product\ [g])} \times 100 \quad (3)$$

$$(Water\ [g]) = (Wet\ product\ [g]) - (Dry\ sample\ [g]) \quad (4)$$

$$(Dry\ sample\ [g]) = (Dry\ weight\ [g]) - (Weight\ of\ pan\ [g]) \quad (5)$$

4.4.3 Ash content analysis

Ash weight analysis was prepared following (Helrich, 1990). Ceramic cups were pre-weighed, and a small amount of each product sample was added to separate cups; around 0.5 g for soup samples, 0.3 g for solid samples and 0.1-0.2 g for emulsions see Appendix B Table 20 for raw data. The samples were put in a furnace, at 550ºC for 6 h. The cups were then weighed, together with their content, and the weight of the ash was calculated.

The mineral content was calculated using Equation 6 and 7.

$$(Mineral\ content\ [\%]) = \frac{(Ash\ [g])}{(Wet\ product\ [g])} \times 100 \quad (6)$$

$$(Ash\ [g]) = (Dry\ weight\ [g] - (Weight\ of\ crucible\ [g]) \quad (7)$$

4.4.4 Texture analysis

Texture analysis was performed for the mayonnaise, fish sausage and the fish balls. Samples of fish sausages and balls were prepared in appropriate sizes, with the height and width of the samples being the same, and the mayonnaise was transferred to a cylindrical tube, until the height reached around 3 cm, see Figure 5. The puncture test was conducted with a 5-mm spherical probe using a 5 kg load cell, at depression a speed of 60 mm/min, using a TA.HDi texture analyzer (Stable Micro Systems, UK) (Abdollahi *et al.*, 2019b). The samples were compressed for 30% of their height. In the case of mayonnaises, a cylindrical probe with 20 mm diameter was used to simulate spreading of the mayonnaise when they are compressed for 30% of their initial height. The force, distance and time were then analyzed, see Appendix B Table 29 and 30 for raw data.

In order to calculate the breaking force and the deformation for the fish balls and the fish sausage Equations 8, 9 and 10 was used.

$$Breaking\ force = Maximum\ force \qquad (8)$$

$$Deformation = Distance\ at\ \max force - initial\ height \qquad (9)$$

The firmness for the mayonnaise was calculated using Equation 10.

$$Firmness = Maximum\ force \qquad (10)$$

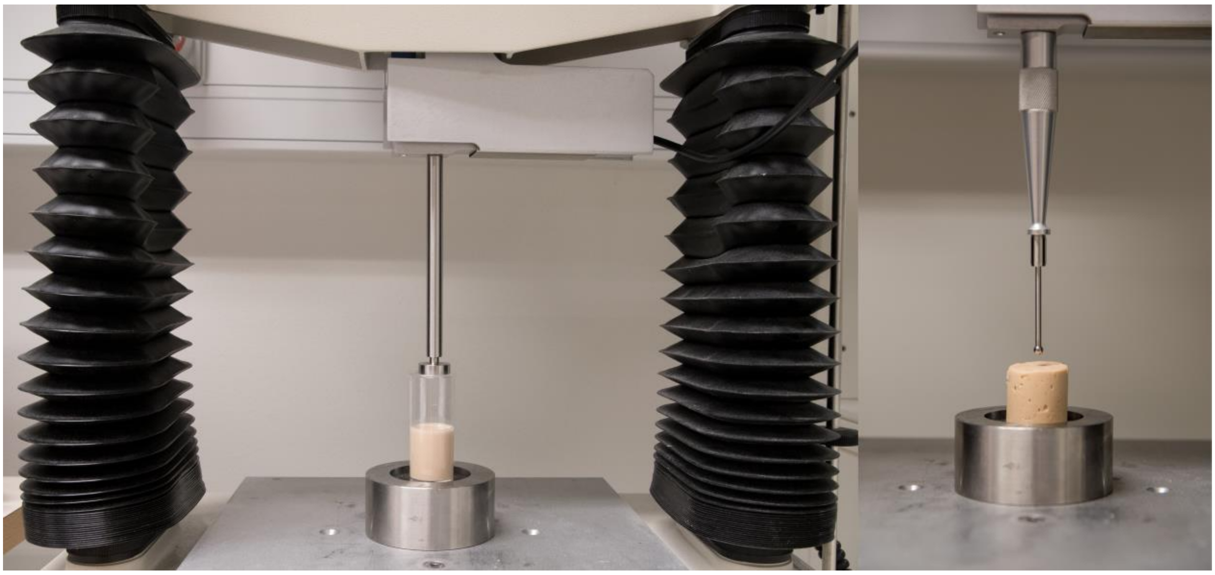

Figure 5 – Representative picture of texture analysis using 20 mm cylindrical probe, for mayonnaises (left), and 5 mm spherical probe, for solid products (right).

4.4.5 Protein measurement using Dumas method

The method for extraction of protein was executed following (Moore *et al.*, 2010). Firstly, the scale was tared with the boats (small containers), and then the sample was weighed in the boat. For the dry samples, of salmon and cod approximately, 0.1 g were measured, see Appendix B, Table 23 for raw data. The boats were then placed in the right order for the machine.

4.4.6 Sensory analysis

To evaluate the different products, eight different panelists five women and three men participated in individual sensory analyses using a Quantitative Descriptive Analysis (QDA) method. This evaluation was important to get an insight whether the product could be interesting for the consumer or not. The panelists were instructed to evaluate several sensory attributes and evaluate them on a scale from 0 to 10. The head attributes were odor, texture and appearance, which also had different sub attributes (Shaviklo *et al.*, 2010). The complete list of sensory attributes that were used for the different products can be found in Appendix A, Table 14. There was no possibility to let the panelists taste the different products due to rules and safety reasons when edible substances are produced in a lab. The different products were marked with a 3-digit random numbers to avoid the panelists to know the real difference between the different products beside from the product type. E.g. the panelists were informed that they evaluated a soup but not what type of soup.

4.4.7 Method for extraction of total fat

The method for extraction of total fat was executed following (Lee *et al.*, 1995) and (Undeland *et al.*, 2002). See appendix B, Table 22 for raw data.

For the extraction of total fat, 2 g of the product of interest and 15 ml of ice-cold chloroform: methanol (1:1) was added to a 50 ml test tube. Thereafter mixed for 15 sec with the Ultra Turrex, IKA Werks, Intermed Labassco at 14000 rpm. To clean the polytron 5 ml chloroform:methanol was added to an empty test tube and ran for 10 sec, then added to the initial 15 ml. (For mayonnaise the relation of chloroform:methanol was 2:1 and for products that do not need to be homogenized, 20 ml chloroform:methanol was added).

8 ml 0.5% NaCl was added to the chloroform:methanol mix and vortexed for 30 sec. Thereafter it was centrifuged for 6 min at 4˚C and 4000xg.

After the centrifugation three clear layers could be seen. To be able to extract 3 ml from the third chloroform layer a glass syringe needle was used to push through the first methanol and water layer and the second muscle layer. This was done twice per test tube and the reweighted tubes were weighed with the chloroform and fat solution.

Thereafter the chloroform was evaporated with a Turbovap evaporator, driven by nitrogen gas, and thereafter weighed again.

The fat content was calculated using Equations 11, 12, 13, 14 and 15.

$$(Fat\ content\ [\%]) = (Factor) \times \frac{(Fat\ [g])}{(Product\ weight\ [g])} \times 100 \qquad (11)$$

$$(Factor) = \frac{10}{(Volume\ of\ chloroform\ [ml])} \quad \text{For 1:1 (all except mayonnaise)} \qquad (12)$$

$$(Factor) = \frac{13.33}{(Volume\ of\ chloroform\ [ml])} \quad \text{For 2:1 (mayonnaise)} \qquad (13)$$

$$(Volume\ of\ chloroform\ [ml]) = \frac{(Weight\ of\ tube, lid, chloroform, fat\ [g]) - (Weight\ of\ empty\ test\ tube\ [g]) - (Weight\ of\ cap\ [g]) - (Mass\ of\ fat\ [g])}{\left(Chloroform\ density\ \left[\frac{g}{ml}\right]\right)} \qquad (14)$$

$$(Mass\ of\ fat\ [g]) = (Weight\ of\ tube\ after\ drying\ with\ nitrogen\ [g]) - (Weight\ of\ empty\ test\ tube\ [g]) \qquad (15)$$

4.4.8 Protein measurement using the Lowry method

Soluble protein content of the products was measured using a modified version of Lowry method (Lowry *et al.*, 1951) and (Markwell *et al.*, 1978). See Appendix B, Table 23 for raw data.

2.5 ml of 1 N NaOH and 2.5 ml of MQ water was added to 1 g solid sample and then homogenized. For the liquid samples, 1 ml was added to 2 ml of 1N NaOH and 2 ml of MQ water and mixed. Thereafter the samples were diluted to contain 10-100 µg/ml and in the last dilution 100 µl sample and 900 µl 0.1 M NaOH to get a 1 ml sample.

Triplicates were made by mixing 333 µl of the sample with 1 ml, a solution made of 800 µl of reagent B (4% $CuSO_4 x 5H_2O$) and 80 ml of reagent A (solution made of 2.0% Na_2CO_3, 0.40% NaOH, 0.16%Na-k-tatarate, 1% SDS). Thereafter the samples were incubated for 30 min at room temperature.

Then, 0.1 ml phenol reagent (1 part Folin-Ciocalteu phenol reagent and 1 part distilled water) was added to the samples, vortexed and incubated at room temperature for 45 min. The samples were then put in the spectrophotometer and read at 660 nm and compared to a standard curve with the equation (NR).

From Equation 16 the protein concentration was calculated, where y is the absorbance and x is the protein concentration.

$$y = 0.004x + 0.0025 \tag{16}$$

4.5 Number of replicates and presentation of results

The results for all tests with replicates are presented in the form of mean ± SD. When measuring dry weight, ash weight and protein content, with LECO, two replicates were used. In the fat content analysis two replicates of two samples from each product were used. The Lowry protein analysis used three replicates and the color analysis used at least five replicates for each sample.

5 Results

5.1 Inventory template

In the inventory template, see Table 2, the products that were produced and the products that were planned to be produced can be seen. More products were investigated in terms of potential of prototypes and the full inventory template can be seen in Appendix A, Table 18. However, fish sticks were never produced due to lack of time and difficulties reproducing factory processes. The soft cheese with shrimps were also rejected because of the complicated factory process. The fish pate was similar to fish balls and fish sausage and was therefore left out. Mayonnaise became a part of the project at a later stage of the project and is therefore not present in the inventory template.

Table 2 - Final list of most promising products to make new products with protein ingredients. Short version of the extensive inventory (Appendix A, Table 18) prepared by investigating the products in terms of their potential as prototype made with protein ingredients.

Product catergory	Product name	Total protein content	Protein sources specifications			Picture of the product
			Name, source and percentage	Functionality (role in the product)	Potential for application of novel proteins	
Restructured seafood product/ fast food	"Archipelago soup"	1.6 g	Shrimp and lobster broth	Taste, nutrition	Medium/high. Would be easy to substitute the broth to our protein powder. The exact amount of every ingredient is specified, hopefully it would not be too hard trying to make this.	(1) 
Restructured seafood product/ fast food	Sliced seafood pate	10.5 g	Salmon 36g Shrimp 26g Egg white Cream Milk	Taste, nutrition, binding, texture.	High. A product that is popular in Sweden and easy to substitute the protein sources.	(2) 
Dairy product	Soft cheese with shrimps	16 g	Hard cheese 58% Shrimp 10% Butter	Texture, nutrition, taste	Medium. Since there already are shrimps in the cheese-spread it would probably not be too hard to use our own shrimp protein. It is also already a popular product on the Swedish market. The challenges will be the process of making the product like pasteurization, temperatures etc. Also, the fact that the exact amount of some ingredients is unknown can be a challenge.	(3) 
Restructured seafood product	Fish sticks	12 g	Alaska pollock 61%	Texture, nutrition, taste	High. As the product is already fish based any of the FPPs could probably be used, in terms of taste and odor. The texture might be the most important parameter to concider.	(4)
Restructured seafood product	Fish balls in stock	5,9 g	Fish meat 56% (Haddock, cod, saithe, greater argentine)	Texture, nutrition, taste, binding.	High. Similarly, to the surimi the product is made from minced fish. The ability of the FPP to be molded into a stable ball will probably be deciding.	(5) 
Restructured seafood product	Salmon sausage	18 g	Salmon 80%	Texture, nutrition,taste	High. Since the sausage only contains meat from fish (salmon). The ability to change the content from salmon mince to our protein will be high and at a good rate of success.	(6) 

1.(Arla, 2019) 2. (Smålandskräftan, 2019) 3. (Kavli, 2019) 4. (Findus Sverige, 2019) 5. (Abba, 2019a) 6. (Korshags, 2019)

In Figure 6 the result of the different products is shown. In Picture 1 all of the fish balls are presented, where the ball on the top is the control ball and the one on the bottom is the one with 100% SPI. In Picture 2 the 100% SPI ball is shown, it was wrinklier than the other balls. The two different fish sausages can be seen in Picture 3, the one with SMB was more reddish than the one with 50% SPI and 50% SMB. In Picture 4 the egg mayonnaise (control) is shown, it was not a perfect result due to the bubbles in the sample. The mayonnaise shrimp had a red color that can be seen in Picture 5. The cod mayonnaise on the other hand had a white color, like the control, but without the bubbles see Picture 6. The difference between the two fish soups is that the shrimp soup, Picture 7, had a more reddish color than the salmon soup Picture 8.

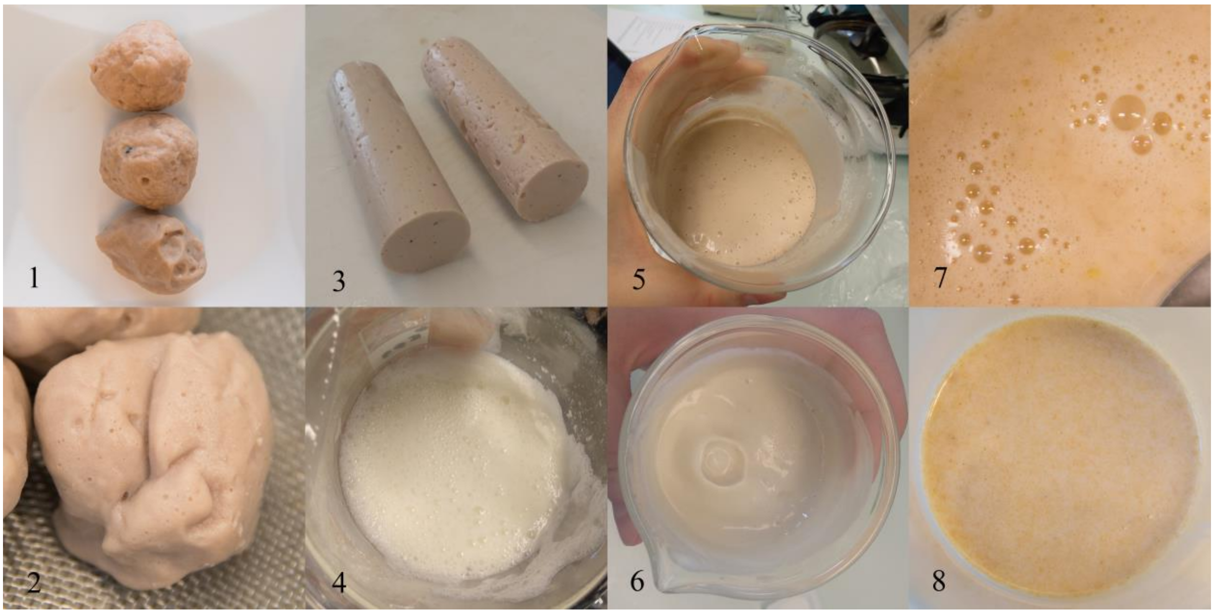

Figure 6 – Finished products. 1. Fish balls with 100% SMB (top), 50% SMB/50% SWI (middle), 100% SPI (bottom) 2. 100% SPI fish ball 3. Fish sausages with 50% SMB/50% SWI (left), 100% SMB (right) 4. Mayonnaise with EWP 5. Mayonnaise with ShPP 6. Mayonnaise with CPP 7. Fish soup with ShPP 8. Fish soup with SaPP

5.2 Dumas method

Table 3 shows the percentage of protein and nitrogen, of the SPI and ShPP, measured with Dumas method.

Table 3 – Results for the Dumas method for protein content in dry samples of the protein isolates.

Sample	Protein (%)
ShPP	54.341±0.553
CPP	81.756±0.319
SaPP	9.841±0.034
SPI	63.533±0.059

5.3 Proximate composition of different products

Moisture, ash, fat and protein content (%) are presented in Table 4-5. For fish balls moisture content varied in the range of 66–74% while fat content was 3.5–14.9% and protein content was found to be between 2.7–13.5%. Fat content and protein content changed significantly when SMB with 100% of SPI in fish balls. The fat content was found to be 14.9% in the control and 3.5% in the 100% SPI and the protein content was 2.7% and 5.5%, respectively. The higher fat content in fish ball control could be due to higher amount of fat in SMB compared to the isolate. The higher amount of protein content in the 100% fish ball can be explained by a higher protein content in SPI compared to the SMB.

The fish sausage moisture varied in the range of 45-49% while the fat content was 30-36%. Protein content varied between 17-25%. The fat content changed when replacing the SMB with SPI where it changed from 36.44% to 30.22%. This change can be explained by the same argument as for the fish balls, that the SMB had a higher fat content than the SPI. On the other hand, the protein content changed in an unexpected way from 25.71% for the control to 17.19% in the 50% sample. This result is unexpected since the SPI had a higher protein content than the SMB. In Table 4 Equation 3-7 and 11-16 were used.

Table 4 - Proximate composition of fish ball made by SMB and 50 and 100% replacement with salmon isolate as well as fish sausage made by 50% replacement of SMB with SPI. Moisture, ash, fat as well as protein content (%). All the analyses were in duplicates except for protein content (%) done in triplicates. The numbers in the product column, for fish balls and sausages, show the percentage of protein isolate used. In the controls, no isolate was used.

Product	Type	Moisture (%)	Ash content (%)	Fat content (%)	Protein (%)
Fish ball	Control	66.45±0.01	2.40±0.05	14.94±1.21	2.70±0.24
	50% SPI 50% SMB	72.77±5.71	2.05*	8.88±0.23	13.57±1.00
	100%	74.17±0.56	2.38±0.10	3.56±0.05	5.54±0.20
Fish sausage	Control	45.09±0.76	3.67±0.12	36.44±0.20	25.71±1.80
	50% SPI 50% SMB	49.19±0.02	3.32*	30.22±0.25	17.19±0.44

* - Single data point

For fish soup none of the compositions varied considerably. Moisture varied between approximately 86-87% while the fat content was 5.6-7.0%. Protein content varied between 0.24-1.42%. The protein content did not differ considerably between the products except that the shrimp soup had a lower protein content of 0.24% while the other two soups had 1.20% and 1.42%. The protein content was low since the product contained a lot of other ingredients such as water and cream.

The mayonnaise moisture varied in the range of 30-34% while the fat content was 54.5-56.5%. Protein content varied between 1-3%. The cod mayonnaise had the lowest content of moisture which was expected since the product became creamier and firmer compared to the other two. The effect was mainly related to the ability of protein to act as emulsifier. The lower protein content in the cod mayonnaise was unexpected since the CPP had the highest protein content compared to ShPP and EWP. In Table 5 Equation 3-7 and 11-16 were used.

Table 5 - Proximate composition of soup made by 100% ShPP and SaPP as well as mayonnaise made by 100% EWP, ShPP and CPP. Moisture, ash, fat as well as protein content (%). All the analyses were in duplicates except for protein content (%) done in triplicates. The numbers in the protein ingredient column show the percentage of protein powder used in these products. Soup control was from the company Arla Foods AB.

Product	Powder type	Moisture (%)	Ash content (%)	Fat content (%)	Protein (%)
Soup	Control	85.94±0.05	1.45±0.05	5.64±0.08	1.20±0.46
	ShPP	86.65±0.80	0.52±0.16	6.64±0.16	0.24±0.03
	SPI	86.90±0.13	-	6.96±0.05	1.42±0.03
Mayonnaise	EWP	32.85*	0.24*	54.6±1.5	2.96±0.50
	ShPP	33.55±0.58	0.24±0.02	54.53±2.85	2.93±0.05
	CPP	30.38±0.01	-	56.53±3.16	1.31±0.13

* - Single data point

5.4 Color measurement

L*, a*, b*, whiteness and redness are presented in Table 6. L* for fish balls varied in the range of 56-67 while a* varied from 4.6-8.1 and b* was found to be in the range of 8.8-14.0. L*, a* and b* for fish balls decreased when replacing more of the SMB with SPI. The whiteness and the redness also decreased in the same order as described above. L* for mayonnaise varied in the range of 72-87 while a* varied from (–1.5)-5.3 and b* was found to be in the range of 7.3-15.0. L* was highest for egg mayonnaise and lowest for shrimp, a* was lowest for egg and highest for shrimp and b* was found to be lowest for egg and highest for shrimp. The whiteness for mayonnaise was found to be highest for egg and lowest for shrimp while the redness was highest for shrimp and lowest for egg. The values for L*, a*, b*, whiteness and redness for cod mayonnaise was always found to be between shrimp and egg. L*, a*, b*, whiteness and redness for the two different fish sausages had no significant differences. The whiteness and the redness for the different products in Table 6 were calculated from Table 21 in Appendix B with Equation 1 and Equation 2, respectively.

Table 6 - Results from the analysis of color and values for the calculated whiteness and redness of the samples. The numbers in the product column, for fish balls and sausages, show the percentage of protein isolate used. In the controls, no isolate was used.

Product	Protein type	L*	a*	b*	Whiteness	Redness
Fish ball	Control	67.00±1.17	8.15±0.33	14.07±0.18	63.20±0.96	0.58±0.02
	50% SPI 50% SMB	64.11±0.98	6.45±0.19	11.98±0.33	61.62±1.00	0.54±0.01
	100% SPI	56.89±1.42	4.64±0.16	8.81±0.63	55.76±1.48	0.53±0.02
Mayonnaise	EWP	87.10±0.08	-1.56±0.02	7.31±0.08	85.09±0.03	-0.21±0.002
	ShPP	72.79±0.16	5.32±0.04	15.01±0.12	68.47±0.10	0.35±0.003
	CPP	82.10±0.12	-0.33±0.02	12.31±0.18	78.28±0.09	-0.03±0.002
Fish sausage	Control	72.20±0.46	6.54±0.09	16.47±0.10	67.03±0.36	0.40±0.005
	50% SPI 50% SMB	71.15±0.14	5.03±0.04	14.68±0.04	67.24±0.12	0.34±0.002

5.5 Texture analysis

The firmness from the different mayonnaise products are presented in Table 7. The firmness varied in the range of 0.05-0.20. When replacing EWP with CPP and ShPP, the firmness changed from 0.104 to 0.201 and 0.057 respectively. The firmness in Table 7 was calculated from Table 30 in Appendix B with Equation 10.

Table 7 – Results of the texture analysis for mayonnaise

Product	Type	Firmness (N)
Mayonnaise	ShPP	0,057±0,0028
	CPP	0,201±0,0021
	EWP	0,104±0,0035

The breaking force and deformation of the different fish balls and fish sausages are presented in Table 8. For fish balls the breaking force varied in the range of 1.07-1.72. When replacing 50% of SMB with 50% SPI, the breaking force of the products increased, but when replacing 100% SMB with 100% protein isolate the breaking force decreased. The deformation also changed significantly when the SPI increased to 50% but then decreased again when 100% SPI was applied. Whereas for fish sausage, when replacing 50% SMB with 50% SPI the breaking force decreased and the deformation increased. The breaking force and the deformation in Table 8 were calculated from Table 29 in Appendix B with Equation 8 and 9, respectively.

Table 8 - Results of the texture analysis for fish balls and fish sausage.

Product	Type	Breaking force (N)	Deformation (mm)
Fish balls	Control	1,48±0,37	7,11±0,93
	50% SPI 50% SMB	1,72±0,32	9,25±0,21
	100% SPI	1,07±0,19	8,65±0,77
Fish sausage	Control	1,20±0,07	5,68±0,23
	50% SPI 50% SMB	1,16±0,05	7,32±0,11

5.6 Sensory analysis

The results from the sensory analysis are presented in Figure 7, 8, 9 and 10. Regarding odor, number zero on the scale was representing a very low intensity of a sensory attribute, compared to 10 which was representing a very high intensity of a sensory attribute. When it came to texture and appearance, a high number was representing how close to perfect the sensory attribute was, rather than how intense the attribute was, e.g. how close the homogeneity of a sausage was to the homogeneity in an ideal sausage product. The exception was the attribute “wrinkle”, see Figure 7 and 8 that got a high grade if the product was very wrinkly. In the attribute “white/red”, see Figure 10, a high grade represented a red color and a low grade represented a white color. The mean values and standard deviations for all sensory attributes and products can be found in Appendix B, Table 25, 26, 27 and 28.

According to Figure 7, the control fish ball and the fish ball with 50% SMB and 50% SPI had overall high and similar scores. The fish ball made from 100% SPI had slightly lower scores, especially the colors. It was also clear that all fish balls had very similar scores for the odor. According to Figure 8, the homogeneity in texture and appearance had higher scores for the fish sausage that consists of 50% SMB and 50% SPI. The same sausage also had lower rancidity than the control sausage with only salmon mince. The sausage including isolate was evaluated to look less appealing than the other one.

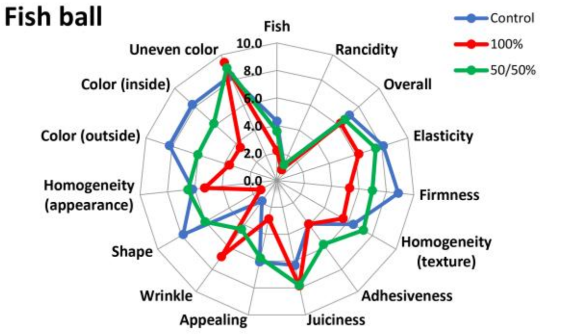

Figure 7 - Results for sensory attributes in fish ball. The control consists of 100% SMB, 100% stands for 100% SPI and 50/50% consists of 50% SMB and 50% SPI.

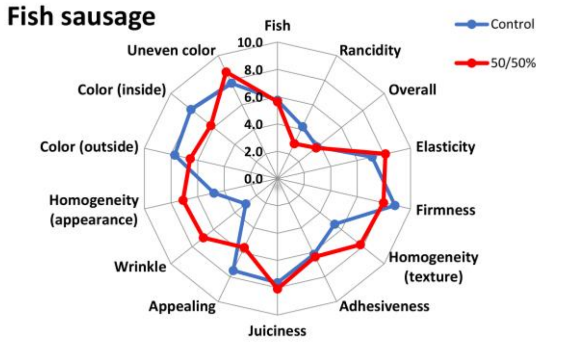

Figure 8 - Results for sensory attributes in fish sausages. The control consists of 100% SMB and 50/50% consist of 50% SMB and 50% SPI.

Both fish soups had low homogeneity for both appearance and texture, which can be seen in Figure 9. The salmon soup had a low rancidity, less smell of fish and a higher overall odor score. It was also evaluated to look more appealing and had slightly higher scores for color. The egg (control) and shrimp mayonnaise had both noticeable lower firmness than the cod mayonnaise, according to Figure 10. The shrimp mayonnaise had a more reddish color. All the mayonnaises had overall high scores and low rancidity.

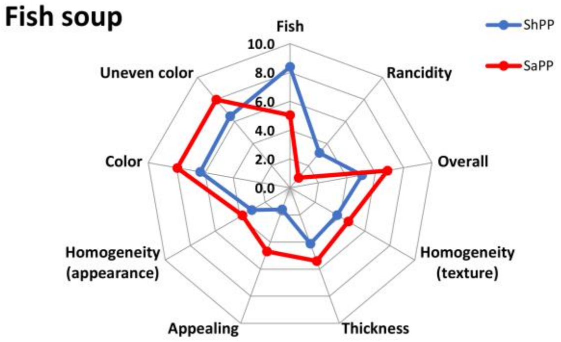

Figure 9 – Results for sensory attributes in fish soups.

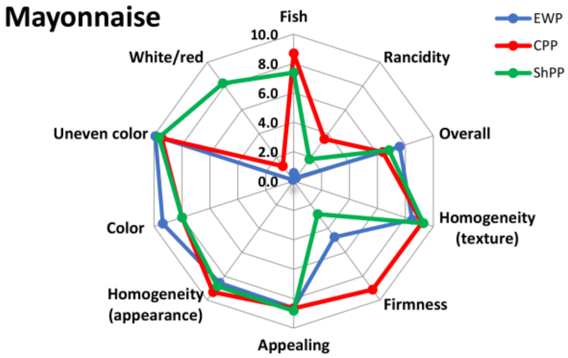

Figure 10 – Results for sensory attributes in mayonnaises.

6 Discussion

The fish soup was easy to produce, but according to Table 5 it had a low content of protein and therefore it is probably not the most promising product to introduce to the market. It would be more essential to apply a product where it is possible to replace a larger amount of protein. According to the sensory test, see Figure 9, the soups had a low homogeneity, for both appearance and texture. The low score of homogeneity might have been due to the cross-linking, after freezing and thawing, which reduced the protein solubility (Abdollahi *et al.*, 2019a). The acidic wine could have increased the positive charge of the protein side chains, preventing it from solubilizing in the fat rich soup. The salmon soup had a low rancidity, which was promising for a future product. The shrimp soup on the other hand did not get a promising result, since it had a high score of rancidity.

The fish ball with 50% SMB and 50% SPI scored similarly, in the sensory test, to the control, with 100% SMB, regarding appeal, see Figure 7. It could have been possible to replace an even higher percentage of SMB with SPI, to obtain an even more environmentally sustainable product. There was a clear difference in structure, according to the sensory test, Figure 7, between the control and the fish ball with 100% SPI. The fish ball with isolate was looser before the cooking, see Figure 2, which could have been due to the changes in protein structure after being recovered using pH-shift method. The changes in protein structure also resulted in a more uneven and wrinkly structure. The 100% SPI fish ball could be improved by adding some polysaccharides, such as carrageenan or starch, or crosslinking enzymes, like microbial transglutaminase and protease inhibitors, such as bovine albumin or egg white albumin (Park, 2013). According to Table 4, it was shown that a higher amount of isolate resulted in a lower percentage of fat. Salmon is a very fatty fish and not usually used for production of fish balls. Normally fish balls are made of white fish like cod, which can be seen in the recipe, Appendix A Table 2 and 3. According to the sensory test, the color of the 100% SPI was not preferred over the color from 100% SMB. The color test also showed that the whiteness of the products decreased with a higher percentage of SPI. The panel concluded that the darker, more brownish color, see Figure 6, of the fish balls with higher percentages of SPI was the reason the color of the control was preferred, over the fish balls containing SPI. The control and the fish ball with 50% SMB and 50% SPI had a more reddish color, see Table 6, giving them an appealing, pinkish hue, which can be observed in Figure 6. However, the sensory test showed that the isolate did not affect the odor of the product, which is promising for future development. According to the texture analysis, the breaking force of the fish ball with 50% SMB and 50% SPI increased, compared to the control, which can be seen in Table 8, while the breaking force was lower for the 100% SPI fish ball. The lower breaking force explained why the fish ball with 100% SPI scored lower for firmness, in the sensory test, see Figure 7, compared to the control, while the increase explained why the fish ball with 50% SMB and 50% SPI scored lower, in firmness, than the control. The same pattern was shown regarding the deformation, which increased in the fish ball with 50% SMB and 50% SPI and decreased in the 100% SPI fish ball. The firmness could be improved by adding additives, as mentioned above.

The mayonnaise made with ShPP got a reddish color, which can be seen in Table 6. This contrasts with the mayonnaise that is available on the market. Despite of this, this result does not have to be a negative aspect, since a reddish color could be expected in products containing shrimp. In the sensory analysis it was clear that the control mayonnaise, made with EWP, and the shrimp mayonnaise were too thin and watery. The cod mayonnaise had a preferable thickness, see sensory attribute "firmness" in Figure 10. All mayonnaises were homogenous, but the egg mayonnaise contained air bubbles. Overall the results from the fish mayonnaises were promising, according to Figure 10, especially the one containing CPP, due to its thickness. Consequently, both shrimp and cod mayonnaise were deemed promising for future studies. The protein content in the product with CPP was lower than expected, see Appendix B, Table 4. This could depend on the fact that the samples had been frozen, and were not completely homogenous, when performing the Lowry protein determination. According to the texture analysis, the firmness was higher for the CPP mayonnaise, compared to the mayonnaises with EWP and ShPP, which can be seen in Table 10. This result to the result in the sensory test, where the cod mayonnaise was clearly firmer. The firm structure observed in the mayonnaise produced from cod could be due to the higher amount of high molecular weight proteins in cod protein isolate, compared to egg and shrimp proteins, which can form a well-structured emulsion system in micro level, which is seen as higher macrostructural firmness (Abdollahi *et al.*, 2018).

According to Table 4, the fish sausage with 50% SMB and 50% SPI had a higher moisture content, compared to the control. This could be explained by changes in protein structure for the SPI after being recovered, using the pH-shift method. The high ash weight, seen in Table 2, for fish sausage could be caused by the fish sausage having a high salt content. Table 2 also showed that the protein content was lower for the sausage with 50% SPI than the control sample. The color analysis, see Table 4, showed that the two different sausage samples had similar whiteness and redness values, indicating that the color for the samples were similar. The sensory analysis for fish sausage, see Figure 8, showed overall high scores. The homogeneity for the texture and the appearance was shown to be better for the sausage containing the SPI. The rancid smell was also lower for the sausage containing the SPI. The only negative result for the sausage with 50% SMB and 50% SPI was that the color was slightly greyer than the control, which was perceived as less appealing. The slight color difference between the two sausages can be observed in Figure 6. According to the texture analysis, the breaking force was similar for both the control and the sausage with 50% SMB and 50% SPI, which can be seen in Table 8. There was a small difference between sausages, regarding the deformation, where the sausage with 50% SMB and 50% SPI had a higher value compared to the control. The similarity between the two sausages, regarding the breaking force, is promising for future product development.

The temperature was not ideal for the products when the sensory test was performed, thus the smell was probably affected by it. When the sensory test was performed, some of the guidelines and parameters were unclear, and could therefore be interpreted differently by the people on the panel. By the time the Lowry protein determination was performed the mayonnaise samples had been frozen, and the protein were therefore defective. This could be a source of error in the later calculations. All the samples were frozen and thawed often, which affected the freshness of the products. Two different types of methods were used for the protein determination in the same calculations. If the LECO machine, used for the Dumas method, had been working all the time it would have been a more reliable result.

More knowledge is needed to be able to produce better products and to understand how the proteins react in different conditions. More tests need to be performed to acquire knowledge about the amount of protein that can be exchanged with isolate and powder and what additives that can be added for a positive effect on the products. There is also a need to test products that we did not have the time or the right equipment to produce, such as fish sticks and soft cheese with shrimp. It could also be desirable to repeat the already preformed production, to be more confident in the results. When more knowledge is gained, and more tests have been performed, it is important to produce the products in a non-laboratory environment, to be able to taste them and evaluate the texture and flavor.

7 Conclusions

Based on the results and the analyzes, a conclusion can be drawn that it was possible to implement the recovered fish proteins from seafood processing side streams in different types of food products. Some products were more promising, such as the fish balls and sausages, regarding texture and color. However, problems regarding texture and color may occur with a complete replacement of SMB with FPI and FPP. The mayonnaises were also promising, especially the one with CPP, since it got the best results, regarding the texture, of the three mayonnaises. The least promising product was the fish soup, due to low protein content and low scores in the sensory analysis.

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Appendix A

As presented below the percentage of protein for the different types of proteins can be seen.

Table 1 - Nutrient content for the different products per 100 g.

Product	Brand	Protein (g)	Fat (g)	Fiber (g)	Salt (g)	Carbonhydrate (Sugar) (g)
"Archipelago-soup"	Kelda (1)	1.6	5.0	-	1.1	6.2 (2.2)
"Shrimp Cheese"	Kavli (2)	16.0	16.0	-	3.4	0
Sliced seafood pate	Smålandskräftan (3)	10.5	11.0	-	1.3	3.7 (1.0)
Fish sticks	Findus (4)	12.0	9.0	0.8	0.8	18.0
Fish balls	Abba (5)	5.9	2.3	-	1.5	5.9
Salmon Sausage	Korshags (6)	18.0	15.0	-	1.7	12.0

1. (Arla, 2019) 2. (Kavli, 2019) 3. (Smålandskräftan, 2019) 4. (Findus Sverige, 2019) 5. (Abba, 2019a) 6. (Korshags, 2019)

Recipes

Fish balls

A prototype that will be produced is fish balls inspired from "fish balls in stock" from Abba. Fish balls is a widely used product in Sweden. The fish balls are made of fish fillet together with some flavoring stock, which can be used to cook your own sauce. The recipe used is inspired from (Spisa.nu, 2019) published on their website. The following table shows the ingredients of the product and the amount to make 2 portions.

Table 2 – The ingredients and their amount to make 2 portions of fish balls.

Ingredients	Amount
Fish fillet (cod)	200 g
Milk	1 tbsp
Potato flour	1 tbsp
Salt	1 tsp

Table 3. Percentage of protein for the different samples.

Sample	Percentage of protein (%)
Fish ball Control	15.5
Fish ball 100% SPI	54.2
Fish ball 50% SMB 50% SPI	34.7

Mix all the ingredients in a food processor or mixer until the mixture becomes fully emulsified (Chen *et al.*, 2011). Then form balls and put them in a bath of water with temperature of 40-45℃ for 20 minutes in order to obtain gelatinization. Thereafter, let the fish

balls cook for 2 minutes in water below 100°C. Then let the fish balls cooling in tap water for 2-5 minutes and then also cooling in iced water for 5-10 minutes.
In this prototype, an appropriate amount of fish meat will be substituted with our fish protein which will be determined in the lab.

Processing method

General ingredients for fish balls besides fish meat are 3-5% salt, 3% starch, 3% sugar, 1% monosodium glutamine (MSG) and 40% water (Park, 2013). Park further described a typical processing method begin with raw material such as *surimi* and fish fillets or minced fish meat. The fish meat is usually frozen which means that before manufacturing takes place, thawing is necessary. Next step is chopping and mixing where all ingredients are added. When chopping *surimi,* a high-speed vacuum cutter or a silent bowl cutter is used. Next step is forming where a machine forms the paste into a ball shape and the balls are then transferred into a setting tank with water with a temperature of 30-40°C. Setting is necessary because it helps the balls to keep the shape and makes the balls more translucent in appearance. Next step according to Park is cooking where the fish balls are cooked in hot water at 90°C for 10 min. Then the fish balls are cooled in room temperature before storing in a cold room at 4-5°C. Fish balls are often traded as a chilled product and therefore usually a freezing process takes place in -18°C before packaging and consumption. Park wrote that the fish balls can also be fried in order to obtain a better taste and gold brown color. The final product can be packed and delivered in an appropriate container either as not cooked but set which are packed in chilled water, brine or broth, but also delivered as cooked and frozen.

Fish mayonnaise

Table 4 - Protein percentage for the fish mayonnaises.

Sample	Percentage of protein (%)
Shrimp mayonnaise	1.62
Cod mayonnaise	2.43
Egg mayonnaise	1.8

Table 5 - Amount of ingredients for each of the samples of mayonnaise.

Sample/Amount of ingredients	EWP	ShPP	CPP
Water	64.01 g	63.87 g	64.54 g
Protein	6.4 g	6.06 g	6.05 g
Lemon	2 ml	2 ml	2 ml
Sunflower oil	130.5 g	129.2 g	129.9

Fish sausage

Table 6 - Protein percentage for fish sausage.

Sample	Percentage of protein (%)
Fish sausage control	13.0
Fish sausage 50% SMB 50% SPI	29.5

Two different fish (salmon) sausages were made. One control and one prototype. The control was made only with the salmon mince while the prototype was made with 50% salmon mince and 50% salmon protein isolate. The two sausage products had the following ingredients shown in Table 7.

Table 7 - Proportion, in gram, of the different ingredients in the two fish sausages.

Fish sausage	Control (g)	Prototype (g)
SMB	139.7	69.8
SPI	-	70.1
Salt	3.9874	3.9919
Vegetable oil	40.0	40.1
Potato starch	3.5162	3.5771
Sucrose	3.0525	3.0303
Tripolyphosphate	1.4930	1.4988

The first step differed for the two sausages. For the control, only salmon mince was put to chop for 40 seconds. Whilst for the prototype, both the salmon mince and the protein isolate, in their 50/50 proportion, were put in the chopper for 1 minute. After the first step, salt was added to the mixes and put in the chopper for 2 minutes equally for the two sausages. Then, the vegetable oil was added to the mixes and again put in the chopper for 2 minutes equally per product. Lastly, Potato starch, sucrose and tripolyphosphate were added to the two mixes and put in the chopper for 3 minutes equally. The two mixes chopped for 3 minutes with a pause of 10 seconds between each minute to gather all the batter on the wall of the chopper and put it in the middle where the chopping occurs. The two batters were then stuffed, with a stuffer and a funnel, into two different plastic sausage casings. The batters in each casing were compressed tightly before the knots at each end were tied.

The two sausages were later incubated at 40°C for 30 minutes. After the incubation, the sausages were cooked at 80°C for 15 minutes. As a final step for the preparation of the sausages, the two products were put into cold water with ice for 15 minutes.

Fish sticks

Fish sticks is a very common everyday food product that is especially popular with kids and families. The fish sticks are made from frozen blocks of either fish mince (cheaper) or fish fillets (more expensive) that are coated in flour, batter and breadcrumbs, after which they are deep fried.

Fish sticks content: Alaska pollock (Theragra chalcogramma) 65 %, wheat flour, rapeseed oil, water, potato starch, salt, spices (paprika, turmeric) and yeast (Findus Sverige, 2019).

Breading content: Wheat flour, wholemeal rye flour, rapeseed oil, yeast, sugar, poppy seeds, salt, rye flour, malted barley (Wasabröd, 2019).

Processing method

Blocks of fish mince, that have been previously blast frozen, on board the fishing boat or directly within landing (The Codex Alimentarius), are cut into rectangular sticks. The sticks then travel through a flow of batter, made from flour, potato starch, water and spices; afterwards the batter-covered sticks are covered in a layer of bread crumbs. In a so called "4-pass" process the sticks go through a four-step process, in order to be covered in batter and breading (Jensen, 2009). First the sticks pass through a flow of water, thawing the outermost layer and wetting the fish; this allows for the flour, that the sticks are covered in, in the second step, to more easily stick to the surface. In the third step the sticks are covered in batter, followed by the fourth step, where they are finally covered in bread crumbs (The Codex Alimentarius).

The battered and breaded products are often pre-fried in rapeseed oil; this stabilizes the product, adds color and removes water, letting the product absorb oil instead, which can reduce or remove the consumers' need for oil in the secondary cooking (Mallett, 1993). Mallett writes that the coating layer also works as a physical protection, for products made from soft and deformable material, like fish mince, as well as a moisture barrier, protecting the product from dehydration during the freezing process. In the frying process the sticks are cooked for just long enough to cook the outside coating, while keeping the inside frozen. The finished fish sticks are then re-frozen and packaged (Sortwell, 1980).

Recipe

Table 8 - Ingredients for the fish sticks.

Ingredients	Amount
Fish mince/filet	200 g
Flour (wheat)	To cover sticks
Oil (rapeseed)	For frying
Breading (Wasa)	To cover sticks
Batter	
Flour (wheat)	50 g
Potato starch	12 g
Water (cold)	100 ml
Salt	1.6 g total (including breading)
Paprika powder	Seasoning (in proportion to salt)
Turmeric powder	Seasoning (in proportion to salt)

1. Add the fish filet and fish powder to a mixer and pulse a few times, until the material is minced.
2. Put the fish mince in a container and press down on it, to form a block.
3. Put the fish mince block in the freezer (-18°C) until it is frozen solid.
4. Prepare two plates for coating, with flour and breading respectively.
5. Prepare a batter mixture from flour, potato starch, salt, spices (paprika powder and turmeric) and cold water.
6. Take the block of fish mince and cut into sticks (around 1x2x8 cm).
7. Dip the frozen fish sticks in hot water, letting the surface thaw. Lightly pad with a paper towel, to remove excess water.
8. Turn the sticks in flour until fully covered.
9. Dip the sticks in the batter mixture, prepared earlier.
10. Turn the battered sticks in breading.
11. Fry the sticks in rapeseed oil (175°C) for a short time (about 30 s), cooking the layer of breading but keeping the fish frozen.
12. Freeze the finished product.
13. Bake in oven at 225°C for 10-12 min or fry in a pan, with oil or butter, on medium heat for about 6 min.

Fish soup

One of the prototypes that will be produced is a soup inspired by the "archipelago soup" by Kelda (Arla, 2019). It is a creamy soup with a taste of white wine, fish and seafood. In the following table the ingredients for the archipelago soup are specified, it is a recipe made for two persons.

Table 9 - Ingredients for the fish soup.

Ingredients	Amount
Shrimp and lobster broth*	3 ¾ dl
Milk	1 ¼ dl
Cream	¾ dl
Potato starch	1 tbsp
White wine extract	10 g
Onion	2 tbsp
Fennel	2 tbsp

* The shrimp and lobster broth are made of water, salt, sugar, natural aromas, shrimp powder, shrimp extract, lobster extract, leek extract and pepper.

Table 10 - Protein percentage for soup.

Sample	Percentage of protein (%)
Shrimp soup	0.93
Salmon soup	0.13
Control soup	1.6

For this prototype the broth will consist of fish protein instead of the shrimp powder, shrimp extract and lobster extract. A specific amount of the fish protein will be determined during the laboration.

Cooking method:

1. Do the broth. *
2. Chop the onion and fennel, and let it fry in the pan.
3. Add milk, cream, white wine extract and the broth, let it boil for some minutes.
4. Add potato starch, the soup should be creamy.
5. Mix the soup to a creamy consistence.

* The broth was switched out to shrimp powder, water, salt, sugar and pepper. Boil it all together before it is added to the soup.

Seafood pate

One popular product in Sweden, especially around Christmas, is seafood pate. With inspiration from liver-, chicken-, seafood- and salmon pate we want to do a prototype similar to these food products. Seafood pate have a high content of salmon and shrimp which is the main ingredient and contributes to taste and texture. Cream contribute to a creaminess and egg binds the different ingredients together.

Table 11 - Ingredients for the seafood pate.

Ingredients	Amount for 2 portions
Fish fillet of haddock and salmon	200 g
Salt	2/3 tsp
White pepper	2/3 pinch
Cayenne pepper	A small pinch
Potato flour	2/3 tbsp
Egg	1 egg
Double cream	1 dl
Crayfish tail and shrimp	67g
Dill	2/3 tbsp

The ingredients in this recipe is almost the same as in the product Seafood pate from Smålandskräftan (Smålandskräftan, 2019). In the laboratory we will replace the fish fillet, crayfish and shrimp, or only a part of it to our fish protein from side streams.

Seafood pate is very easy to make, all ingredients except the double cream, crayfish tail and shrimp are blended in a mixer (Journal, 2019). Then add the double cream and carefully blend the crayfish tail and shrimp into the mix. Put the fish mix in a baking tin and bake for 45 minutes on 175 degrees in the oven in a water bath.

Soft cheese with shrimps

Soft cheese used as cheese-spread are a common and popular Swedish dish, used mostly as a sandwich-spread but can also be used in cooking. The product that will be inspiration for this prototype is the soft cheese from Kavli, with pieces of shrimps in it (Kavli, 2019). In the following table, the ingredients of the initial product are specified but the amount of water, food additives, butter and preservatives are unknown.

Table 12 - Ingredients for the soft cheese with shrimps.

Ingredients	Amount
Hard cheese	58 %
Water	-
Shrimps	10 %
Food additives (E339, E452)	-
Butter	-
Preservatives (E202, E234)	-

What will be the difference between this product and the prototype are the shrimps that will be exchanged with shrimp protein. The exact amount of shrimp protein may therefore change for the final prototype.

Table 13 - Sensory attributes tested, for the different product groups.

Sensory attribute		Fish ball	Fish sausage	Fish soup	Mayonnaise
Odor	Fish	×	×	×	×
	Rancidity	×	×	×	×
	Overall	×	×	×	×
Texture	Elasticity	×	×		
	Firmness	×	×		×
	Homogeneity	×	×	×	×
	Adhesiveness	×	×		
	Juiciness	×	×		
	Thickness			×	
Appearance	Appealing	×		×	×
	Wrinkle	×			
	Shape	×			
	Homogeneity	×		×	×
	Color (outside)	×			
	Color (inside)	×			
	Color			×	×
	Uneven color	×		×	×
	White/red				×

Table 14 - The weights of the fish balls with 100% SMB before and after cooking.

Fish balls (100% SMB)	Weight before cooking (g)	Weight after cooking (g)
1	23.4	22.1
2	21.5	21.4
3	19.8	19.0
4	18.8	18.5
	Total = 83.5	

Table 15 - The weights of the fish balls with 50% SMB and 50% SPI before and after cooking.

Fish balls (50% SMB+ 50% SPI)	Weight before cooking (g)	Weight after cooking (g)
1	21.9	21.4
2	21.6	18.6
3	21.7	21.3
	Total = 76.9	

Table 16 - The weights of the fish balls with 100% SPI before and after cooking.

Fish balls (100% SPI)	Weight before cooking (g)	Weight after cooking (g)
1	21.7	15.6
2	23.5	17.9
3	21.4	19.3
4	22.8	16.7
	Total = 96.4	

Table 17 - Proportion, in gram, of the different ingredients in the two fish sausages.

Fish sausage	Control (g)	Prototype (g)
SMB	139.7	69.8
SPI	-	70.1
Salt	3.9874	3.9919
Vegetable oil	40.0	40.1
Potato starch	3.5162	3.5771
Sucrose	3.0525	3.0303
Tripolyphosphate	1.4930	1.4988

Table 18 – Inventory template, for all the different products considered.

Product catergory	Product name	Total protein content of product	Protein sources specifications		
			Name, source and percentage	Functionality (role in the product)	Potential for application of novel proteins
Vegetarian substitute of meat	Soy sausage (1)	18 g	Plant based: wheat (35 %), soy (18 %)	The soy is used to substitute protein, from meat. Can also sometimes be added to reduce cost and increase moisture. Emulsifying agent (high fat) Gel product	Medium/high. In some countries they already eat a lot of fish sausage and since sausage usually is made from meat, it will probably not be as hard to convince people that eat meat as a vegetarian/vegan.
Vegetarian substitute of meat	Soy Chorizo (2)	14 g	Plant based: wheat (18.5 %), soy (13 %)	The soy is used to substitute protein, from meat. Can also sometimes be added to reduce cost and increase moisture.	Same as for soy sausage, above.
Vegan substitute of meat	Vegan nuggets (3)	14 g	Plant based: soy (45 %)	The soy is used to substitute protein, from meat. Can also sometimes be added to reduce cost and increase moisture.	Medium/high. To switch from chicken to fish should not be a problem, as long as we can make the product as tasty.
Canned food, processed meat	Chicken paté (4)	10 g	Animal source: chicken (29 %), milk protein (3 %)	Texture, nutrition, taste.	Medium/high. There is already paté from fish.
Dairy	Cheese-spread (5)	15 g	Animal source: hard cheese (45 %), bacon (2.9 %), pork meat (2.9 %)	Cheese is what the product is made of basically, the rest of the protein is probably mainly for the taste.	Medium. There are already cheese-spreads with shrimp, so maybe fish could also be an alternative.
Bakery/ cooking produc t	Breadcrumbs /raspings (6)	11.5 g	Plant based: wheat (uncertain amount)	It is used for breading different types of foods. It is also used for thickening stews and to bind the fat in meat loafs and meat balls, to make them hold together.	Low. Might be hard to convince the consumer to bake with fish protein. In cooking with meat, it might be easier.
Vegetarian substitute of meat	Oumph (7)	17 g	Plant based: soy 23%	It is the main source of protein, used as a meat substitute.	Medium. The problem is that vegetarian products and meat substitutes are primarily targeted for people who don't eat meat for ethical and climate reasons. With that said I think we will lose some of the audience, due to the ethical reasons.
Vegetarian substitute of meat	Vegan burger (8)	15 g	Plant based: soy 21%	It is the main source of protein, used as a meat substitute.	Same as for oumph, above.
Restructured seafood product	Shrimp-soup (9)	8.1 g	Fish, milk protein (uncertain percentage)	Fish is the main source of protein, probably added for the nutrition and taste.	Medium/high. Easy to substitute, no need to worry about the taste.
Processed meat product	Liver pate (10)	11 g	Animal source: pig liver 31%	Nutrition and taste.	High. There are products similar to this one made of fish, for example fish pate.
Bakery/cooking product	Panko - flakes from bread crumbs (11)	13 g	Plant based: wheat (uncertain percentage)	Absorb fat and to stick to different food surfaces, for example as a breading for fish.	Medium. Possibility to make these flakes from dried fish.
Processed meat product	Corned beef (12)	21 g	Animal source: beef and intestines from livestock 98,5%	Texture, nutrition and taste.	Medium. High possibility to make the same product from fish protein, but the texture might not be the same.
Restructured seafood product	Fish sticks (13)	12 g	Animal source: Alaska pollock 61%	Texture, nutrition, taste. Breading (texture, fat/water retention)	High. As the product is already fish based any of the FPPs could probably be used, in terms of taste and odor. The texture might be the most important parameter to concider.

1. (Hälsans kök, 2019d) 2. (Hälsans kök, 2019b) 3. (Hälsans kök, 2019c) 4. (Argeta, 2019) 5. (Kavli, 2019) 6. (Eldorado, 2019) 7. (Oumph!, 2019) 8. (Hälsans kök, 2019a) 9. (Arla, 2019) 10. (Arbogapastej, 2019) 11. (Taste from Asia, 2019) 12. (Sevan, 2019) 13. (Findus Sverige, 2019)

Table 19 – Continuation of inventory template, for all the different products considered.

Product catergory	Product name	Total protein content of product	Protein sources specifications		
			Name, source and percentage	Functionality (role in the product)	Potential for application of novel proteins
Restructured seafood product	Surimi (14)	8 g	Animal source: Surimi 41% (Alaska pollok, Hake, Hoki) Egg white protein, soy protein	Texture, nutrition, taste, binding.	High. As the product is molded from minced fish product it is ideal as a candidate to substitute the raw material with FPP. The texture and ability to be molded into a stable product will be important. The product also contains soy protein and egg white protein, which have similar properties as the FPPs
Restructured seafood product	Fish balls in lobster sauce (15)	4.1 g	Animal source Balls: Fish meat 56% (Haddock, cod, saithe, greater argentine) Total: Fish meat 28%	Texture, nutrition, taste, binding.	High. Similarly to the surimi, the product is made from minced fish. The ability of the FPP to be molded into a stable ball will probably be deciding.
Restructured seafood product	Fish balls in stock (16)	5.9 g	Animal source: Balls: Fish meat 56% (Haddock, cod, saithe, greater argentine)	Texture, nutrition, taste, binding.	Same as for fish balls in lobster sauce, above
Restructured seafood product	Shrimp stew (17)	4.2 g	Animal source: Shrimp 16%	Taste (nutrition)	Medium. Would probably have to be replaced by products from shrimp, as the taste is, most likely, the primary function of the fish protein.
Vegan substitute of meat	Vego balls (18)	19 g	Plant based: wheat, soy	The soy is used to substitute protein, from meat. Can also sometimes be added to reduce cost and increase moisture. Taste, nutrition, texture.	Medium/high. Since the FPPs share many properties of the soy protein it should be possible to exchange the protein base. The main challenges will be the taste and texture.

14. (Fisherman, 2019) 15. (Abba, 2019b) 16. (Abba, 2019a) 17. (Abba, 2019c) 18. (Anamma, 2019)

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Appendix B

Analysis

Table 20 – Raw data of the ash method analysis.

Weight / Sample	Crucible	Before	After	Water	Dry	Minerals (%)
Fish soup control	33.6248	0.5054	33.6323	0.4979	0.0075	1.483973091
Fish soup control	33.9163	0.5660	33.9243	0.558	0.0080	1.413427562
Shrimp soup	34.5639	0.5435	34.5661	0.5413	0.0022	0.404783809
Shrimp soup	34.4296	0.4919	34.4327	0.4888	0.0031	0.630209392
Fish ball control	5.9665	0.3173	5.974	0.3098	0.0075	2.363693665
Fish ball control	5.2197	0.3075	5.2272	0.3	0.0075	2.43902439
Fish ball 50% SMB 50% SPI	5.7241	0.3073	5.7304	0.301	0.0063	2.050113895
Fish ball 50% SMB 50% SPI	5.7400	0.2975	5.547	0.4905	-0.1930	-64.87394958
Fish ball 100% SPI	5.3057	0.3079	5.3128	0.3008	0.0071	2.305943488
Fish ball 100% SPI	5.7145	0.3101	5.7221	0.3025	0.0076	2.450822315
Sausage control	5.5382	0.3092	5.5498	0.2976	0.0116	3.751617076
Sausage control	5.9142	0.3129	5.9254	0.3017	0.0112	3.579418345
Sausage 50% SMB 50% SPI	6.2820	0.2736	5.2914	1.2642	-0.9906	-362.0614035
Sausage 50% SMB 50% SPI	5.7260	0.2919	5.7357	0.2822	0.0097	3.323055841
Mayonnaise EWP	5.9348	0.1862	5.9345	0.1865	-0.0003	-0.161117078
Mayonnaise EWP	6.0702	0.2089	6.0707	0.2084	0.0005	0.239348971
Mayonnaise shrimp	5.8471	0.1621	5.8475	0.1617	0.0004	0.246761258
Mayonnaise shrimp	5.8803	0.1343	5.8806	0.134	0.0003	0.223380491
Mayonnaise cod	5.5755	0.1903	5.5752	0.1906	-0.0003	-0.157645822
Mayonnaise cod	5.6953	0.1408	5.6933	0.1428	-0.0020	-1.420454545

Table 21 – Raw data of the color analysis.

Product:	L*	a*	b*	Whiteness	Redness
Fish ball control	68.16	8.35	14.07	64.20233248	0.59346
Fish ball control	67.26	8.52	14.3	63.27142802	0.5958
Fish ball control	66.43	7.79	14.16	62.7423216	0.55014
Fish ball control	65.47	7.66	13.75	62.05189069	0.55709
Fish ball control	65.59	8.04	13.94	62.01298511	0.57676
Fish ball control	68.03	8.32	14.15	64.06219539	0.58799
Fish ball control	68.07	8.37	14.15	64.08615448	0.59152
Mean	67.0014286	8.15	14.0742857	63.20418682	0.57897
SD	1.17422923	0.32557641	0.1793374	0.955242478	0.01847
Fish ball 50% SMB 50% SPI	64.96	6.39	11.72	62.50343882	0.54522
Fish ball 50% SMB 50% SPI	64.42	6.49	11.65	62.00290801	0.55708
Fish ball 50% SMB 50% SPI	64.91	6.18	11.88	62.44158017	0.5202
Fish ball 50% SMB 50% SPI	65.23	6.69	12.06	62.59475171	0.55473
Fish ball 50% SMB 50% SPI	62.83	6.47	12.16	60.35992684	0.53207
Fish ball 50% SMB 50% SPI	64.04	6.41	12.15	61.50542636	0.52757
Fish ball 50% SMB 50% SPI	63.94	6.24	11.63	61.60054558	0.53654
Fish ball 50% SMB 50% SPI	62.58	6.71	12.61	59.94637844	0.53212
Mean	64.11375	6.4475	11.9825	61.61936949	0.53819
SD	0.9802323	0.1887364	0.33242615	0.996745666	0.01306
Fish ball 100% SPI	55.76	4.83	9.58	54.47766592	0.50418
Fish ball 100% SPI	55.3	4.91	9.71	53.99475899	0.50566
Fish ball 100% SPI	55.54	4.56	8.94	54.42139976	0.51007
Fish ball 100% SPI	58.15	4.62	8.61	57.02443718	0.53659
Fish ball 100% SPI	58.77	4.55	8.47	57.66377083	0.53719
Fish ball 100% SPI	56.72	4.45	8.06	55.75155935	0.55211
Fish ball 100% SPI	58.01	4.59	8.29	56.95406756	0.55368
Mean	56.8928571	4.64428571	8.80857143	55.75537994	0.5285
SD	1.41614164	0.16450503	0.63354633	1.483305488	0.02155
Shrimp mayonnaise	72.54	5.34	14.91	68.30023186	0.35815
Shrimp mayonnaise	72.71	5.25	14.88	68.47665944	0.35282
Shrimp mayonnaise	72.86	5.32	15.02	68.52806965	0.35419
Shrimp mayonnaise	72.93	5.31	15.11	68.54696994	0.35142

Shrimp mayonnaise	72.91	5.37	15.14	68.5051655	0.35469
Mean	72.79	5.318	15.012	68.47141928	0.35426
SD	0.16416455	0.04438468	0.11606033	0.099232763	0.00252
Cod mayonnaise	82.23	-0.32	12.42	78.31747939	-0.02576
Cod mayonnaise	82.2	-0.34	12.37	78.32115086	-0.02749
Cod mayonnaise	82.1	-0.36	12.13	78.37417054	-0.02968
Cod mayonnaise	82.07	-0.31	12.5	78.14065417	-0.0248
Cod mayonnaise	81.92	-0.32	12.11	78.23670751	-0.02642
Mean	82.104	-0.33	12.306	78.27803249	-0.02683
SD	0.1225969	0.02	0.17615334	0.091156914	0.00187
Egg mayonnaise	87.17	-1.57	7.41	85.10094298	-0.21188
Egg mayonnaise	87.16	-1.56	7.35	85.12311525	-0.21224
Egg mayonnaise	87.14	-1.58	7.33	85.11360017	-0.21555
Egg mayonnaise	87	-1.55	7.21	85.05387676	-0.21498
Egg mayonnaise	87.03	-1.54	7.23	85.0713229	-0.213
Mean	87.1	-1.56	7.306	85.09257161	-0.21353
SD	0.07905694	0.01581139	0.08414274	0.029128994	0.00165
Fish sausage control	71.83	6.48	16.33	66.80047892	0.39682
Fish sausage control	72.69	6.59	16.47	67.43429565	0.40012
Fish sausage control	71.69	6.41	16.45	66.63614681	0.38967
Fish sausage control	72.15	6.59	16.61	66.9100816	0.39675
Fish sausage control	72.66	6.63	16.49	67.39091231	0.40206
Mean	72.204	6.54	16.47	67.03438306	0.39708
SD	0.46128083	0.09165151	0.1	0.359095147	0.00472
Fish sausage 50% SMB 50% SPI	71.26	5.06	14.67	67.33809405	0.34492
Fish sausage 50% SMB 50% SPI	71.06	5.05	14.74	67.13217835	0.34261
Fish sausage 50% SMB 50% SPI	71.25	5.05	14.72	67.30835887	0.34307
Fish sausage 50% SMB 50% SPI	71.24	4.98	14.65	67.34176214	0.33993
Fish sausage 50% SMB 50% SPI	70.94	4.99	14.64	67.08019897	0.34085
Mean	71.15	5.026	14.684	67.24011848	0.34228
SD	0.143527	0.03781534	0.04393177	0.12431082	0.00195

Table 22 – Raw data of the fat content analysis.

Sample	Weight of the product (g)	Weight of empty test tube	Weight of tube with lid + chloroform and fat	Weight of tube when dried	Mass of fat (g)	Volume Chloroform (ml)	Factor	Fat content (%)
Fish ball control	1.955	13.0274	19.0057	13.1081	0.0807	2.997919	3.335647	13.76914
	1.955	13.0124	19.264	13.0996	0.0872	3.17698	3.147643	14.03962
Fish ball control	2.1391	12.9345	19.3549	13.045	0.1105	3.274631	3.053779	15.77498
	2.1391	12.8642	19.3996	12.98	0.1158	3.348255	2.98663	16.1681
Fish ball 50% SMB 50% SPI	1.989	13.0852	18.925	13.1376	0.0524	2.92396	3.42002	9.010007
	1.989	13.041	19.2668	13.0951	0.0541	3.181879	3.142797	8.548281
Fish ball 50% SMB 50% SPI	2.2086	13.2921	18.6429	13.3439	0.0518	2.596174	3.851821	9.033974
	2.2086	13.142	19.2028	13.2025	0.0605	3.066846	3.260679	8.931952
Fish ball 100% SPI	2.0803	13.129	18.0763	13.1465	0.0175	2.348389	4.258238	3.582135
	2.0803	13.2149	19.5291	13.0464	-0.1685	3.390604	2.949327	-23.8889
Fish ball 100% SPI	1.9213	12.9677	18.9546	12.9887	0.021	3.043758	3.285412	3.590988
	1.9213	13.101	19.3187	13.1225	0.0215	3.198322	3.126639	3.498816
Fish sausage control	1.9666	11.6608	17.7442	11.874	0.2132	2.97953	3.356234	36.38508
	1.9666	13.0809	19.5148	13.3112	0.2303	3.203289	3.121792	36.55795
Fish sausage control	2.2466	13.0918	19.4251	13.3455	0.2537	3.120067	3.205059	36.19352
	2.2466	13.1714	20.0217	13.4551	0.2837	3.446913	2.901147	36.6356
Fish sausage 50% SMB 50% SPI	2.0167	12.7994	19.0645	12.9907	0.1913	3.116174	3.209063	30.44051
	2.0167	13.1087	8.3225	13.2581	0.1494	-4.27268	-2.34045	-17.3384
Fish sausage 50% SMB 50% SPI	1.9972	12.9234	19.0249	13.1058	0.1824	3.012349	3.319668	30.31782
	1.9972	13.1638	18.4173	13.3111	0.1473	2.466779	4.05387	29.89861
Control soup	2.0759	13.0075	20.7975	13.0566	0.0491	4.235034	2.361256	5.584936

	2.0759	11.7255	17.7057	11.7608	0.0353	3.029664	3.300696	5.612725
Control soup	2.0788	13.188	20.284	13.2319	0.0439	3.772752	2.650585	5.597493
	2.0788	12.9899	19.3764	13.0294	0.0395	3.29953	3.030734	5.758804
Shrimp soup	2.043	13.1233	20.1076	13.1751	0.0518	3.692483	2.708205	6.866618
	2.043	13.1408	20.5206	13.1937	0.0529	3.957181	2.527051	6.543368
Shrimp soup	2.1215	13.0707	20.0869	13.1219	0.0512	3.714295	2.692301	6.497563
	2.1215	11.9112	19.2954	11.967	0.0558	3.958188	2.526409	6.644996
Salmon soup	1.9857	12.8383	20.2503	12.8938	0.0555	3.977047	2.514428	7.027788
	1.9857	11.6975	18.9348	11.7506	0.0531	3.861409	2.589728	6.925243
Salmon soup	2.1082	11.6445	19.3546	11.7054	0.0609	4.17349	2.396076	6.921594
	2.1082	12.7752	20.5748	12.8373	0.0621	4.232752	2.362529	6.959163
Mayonnaise egg	2.3494	12.7328	19.637	13.1691	0.4363	3.380671	3.056592	56.76306
	2.3494	11.541	18.6455	11.9753	0.4343	3.516443	2.938576	54.32125
Mayonnaise egg	2.1501	12.8565	19.9275	13.2521	0.3956	3.519933	2.935662	54.01367
	2.1501	12.8617	20.3366	13.2805	0.4188	3.775436	2.736991	53.31155
Mayonnaise shrimp	1.9801	13.1177	20.1383	13.4658	0.3481	3.517987	2.937286	51.63726
	1.9801	13.0532	19.6252	13.3802	0.327	3.231074	3.198111	52.81462
Mayonnaise shrimp	2.1827	12.9494	20.13	13.3704	0.421	3.576443	2.889277	55.72848
	2.1827	11.7371	19.228	12.197	0.4599	3.758591	2.749257	57.9275

Table 23 – Raw data from Dumas method.

Result / Sample	Mass (g)	Protein (%)	Nitrogen (%)
SPI	0.1033	63.574	11.393
SPI	0.1125	63.491	11.378
ShPP	0.1001	81.530	14.611
ShPP	0.1020	81.981	14.692

Table 24 - Result of pH before and after lemon was added to the mayonnaise samples.

Sample/pH	EWP	ShPP	SaPP
pH before lemon	6.94	7.1	6.96
pH after lemon	6.56	6.77	6.67

Table 25 - Mean values and standard deviation for fish ball from the sensory test.

	Sensory attributes	Control	100% SPI	50% SMB 50% SPI
Odor	Fish	4.3±3.0	2.2±1.1	3.6±2.6
	Rancidity	1.3±1.6	0.9±1.1	1.2±1.1
	Overall	7.1±1.7	6.2±1.7	6.6±2.0
Texture	Elasticity	8.1±1.8	6.3±2.4	7.6±1.6
	Firmness	8.9±0.5	5.3±2.9	7.0±1.8
	Homogeneity	6.4±2.2	5.6±2.3	7.3±1.4
	Adhesiveness	3.9±1.7	3.9±7.8	5.8±2.4
	Juiciness	6.3±1.7	7.8±1.5	7.8±0.9
Appearance	Appealing	6.1±2.2	2.9±1.4	5.8±2.3
	Wrinkle	1.9±1.2	6.9±1.9	4.4±2.4
	Shape	7.9±1.6	1.4±1.3	6.0±2.7
	Homogeneity	6.2±2.6	5.3±2.8	6.5±1.7
	Color (outside)	8.2±0.8	3.6±1.5	6.0±2.1
	Color (inside)	8.3±0.8	3.6±1.5	6.2±1.9
	Uneven color	8.3±1.8	9.4±0.4	9.0±0.7

Table 26 - Mean values and standard deviation for fish sausage from the sensory test.

	Sensory attributes	Control	50% SMB 50% SPI
Odor	Fish	5.8±2.8	5.6±3.0
	Rancidity	4.2±3.4	2.8±2.7
	Overall	3.7±2.2	3.6±2.6
Texture	Elasticity	7.1±1.9	8.1±1.2
	Firmness	8.8±1.1	8.0±0.9
	Homogeneity	5.4±2.3	7.8±1.1
	Adhesiveness	6.2±2.6	6.4±2.3
	Juiciness	7.6±1.1	8.1±0.7
Appearance	Appealing	7.5±1.1	5.6±2.7
	Wrinkle	3.0±1.6	7.0±2.5
	Homogeneity	4.8±1.4	7.1±0.9
	Color (outside)	7.7±1.2	6.6±1.8
	Color (inside)	8.1±1.0	6.21.1
	Uneven color	7.8±2.1	8.7±1.6

Table 27 - Mean values and standard deviation for fish soup from the sensory test.

	Sensory attributes	Shrimp	Salmon
Oduor	Fish	8.4±1.2	5.0±3.4
	Rancidity	3.2±2.9	0.9±1.1
	Overall	5.1±1.4	6.9±2.3
Texture	Homogeneity (texture)	3.8±3.3	4.7±3.2
	Thickness	4.1±3.0	5.4±3.3
Appearance	Appealing	1.6±0.8	4.7±3.2
	Homogeneity (appearance)	3.1±2.7	3.8±3.3
	Color	6.3±1.8	8.0±1.1
	Uneven color	6.5±1.9	8.0±1.0

Table 28 - Mean values and standard deviation for mayonnaise from the sensory test.

	Sensory attributes	EWP	CPP	ShPP
Odor	Fish	0.6±1.2	8.7±1.2	7.4±2.1
	Rancidity	0.3±0.4	3.5±4.1	1.8±1.8
	Overall	7.6±1.6	6.4±2.5	6.8±1.5
Texture	Homogeneity	8.5±1.2	9.2±0.8	9.3±0.4
	Firmness	4.7±2.7	9.1±1.7	2.8±2.3
Appearance	Appealing	8.6±0.7	8.7±1.5	8.8±1.2
	Homogeneity	8.6±0.8	9.3±0.8	8.8±0.7
	Color	9.4±0.6	8.0±2.7	8.0±1.9
	Uneven color	9.9±0.0	9.5±0.9	9.6±0.3
	White/red	0.1±0.1	1.3±1.3	8.2±0.6

Table 29 - Raw data for the texture analysis for fish balls and fish sausage.

Product	Type	Max Force	Distance at max force (mm)	Time at max force (s)	Initial height(mm)	Breaking force	Deformation (mm)
Fish ball	Control	1.102	7.425	7.395	0	1.102	7.425
	Control	1.832	7.842	7.81	0.001	1.832	7.841
	Control	1.492	6.056	6.035	0	1.492	6.056
	Mean	1.4753333	7.107666667	7.08	0.00033	1.475333333	7.107333333
	SD	0.3652853	0.934330955	0.928479941	0.00057	0.365285277	0.933938078
	50% SMB 50% SPI	2.061	9.414	9.38	0	2.061	9.414
	50% SMB 50% SPI	1.434	9.011	8.975	0	1.434	9.011

	50% SMB 50% SPI	1.663	9.329	9.295	0	1.663	9.329
	Mean	1.7193333	9.251333333	9.216666667	0	1.719333333	9.251333333
	SD	0.3172733	0.212429596	0.213561076	0	0.317273279	0.212429596
	100% SPI	1.207	9.193	9.16	0	1.207	9.193
	100% SPI	0.935	8.103	8.07	0.001	0.935	8.102
	Mean	1.071	8.648	8.615	0.0005	1.071	8.6475
	SD	0.192333	0.770746391	0.770746391	0.00007	0.192333044	0.771453498
Fish sausage	Control	1.251	5.515	5.495	0	1.251	5.515
	Control	1.156	5.841	5.82	0	1.156	5.841
	Mean	1.2035	5.678	5.6575	0	1.2035	5.678
	SD	0.0671751	0.230516811	0.229809704	0	0.067175144	0.230516811
	50% SMB 50% SPI	1.184	7.204	7.175	0.001	1.184	7.203
	50% SMB 50% SPI	1.186	7.344	7.315	0	1.186	7.344
	50% SMB 50% SPI	1.102	7.425	7.395	0	1.102	7.425
	Mean	1.1573333	7.324333333	7.295	0.00033	1.157333333	7.324
	SD	0.0479305	0.11180489	0.111355287	0.00057	0.04793050	0.112343224

Table 30 – Raw data for texture analysis for mayonnaise.

Product	Type	Max Force	Distance at max force (mm)	Time at max force (s)	Initial height (mm)	Breaking force
Mayonnaise	ShPP	0.059	3.429	3.415	0	0.059
	ShPP	0.055	3.96	3.945	0	0.055
	Mean	0.057	3.6945	3.68	0	0.057
	SD	0.0028284	0.375473701	0.374766594	0	0.002828427
	CPP	0.202	5	4.985	0	0.202
	CPP	0.199	4.982	4.965	0	0.199
	Mean	0.2005	4.991	4.975	0	0.2005
	SD	0.0021213	0.012727922	0.014142136	0	0.00212132
	EWP	0.101	5	4.985	0	0.101
	EWP	0.106	4.646	4.63	0	0.106
	Mean	0.1035	4.823	4.8075	0	0.1035
	SD	0.0035355	0.250315801	0.251022907	0	0.003535534

Table 31 - Raw data for the dry weight analysis.

Sample	Pan	Before	After 1	Water 1	Dry 1	Percent water 1
Fish soup control	1.0787	2.3272	1.4066	1.9993	0.3279	85.91010657
Fish soup control	0.9712	2.3473	1.3003	2.0182	0.3291	85.97963618
Salmon soup	18.4498	2.7163	18.808	2.3581	0.3582	86.81294408
Salmon soup	18.0326	2.6872	18.3822	2.3376	0.3496	86.99017565
Shrimp soup	29.306	3.0662	29.698	2.6742	0.392	87.21544583
Shrimp soup	29.4159	2.6481	29.7845	2.2795	0.3686	86.08058608
Fish ball control	29.416	2.4341	30.2348	1.6153	0.8188	66.36128343
			30.2329	1.6172	0.8169	66.43934103
Fish ball control	29.305	2.2107	30.0476	1.4681	0.7426	66.40882978
			30.0465	1.4692	0.7415	66.45858778
Fish ball 50% SMB 50% SPI	18.0299	1.7405	18.5747	1.1957	0.5448	68.69864981
			18.5741	1.1963	0.5442	68.73312267
Fish ball 50% SMB 50% SPI	15.8471	2.294	16.3806	1.7605	0.5335	76.74367916
			16.3792	1.7619	0.5321	76.80470793
Fish ball 100% SPI	18.449	1.8455	18.9204	1.3741	0.4714	74.45678678
			18.9184	1.3761	0.4694	74.56515849
Fish ball 100% SPI	18.2347	1.9037	18.7364	1.4020	0.5017	73.64605768
			18.7341	1.4043	0.4994	73.76687503
Sausage control	1.3592	2.7598	2.8598	1.2592	1.5006	45.62649467
Sausage control	1.0563	2.033	2.1836	0.9057	1.1273	44.54992622
Sausage 50/% SMB 50% SPI	1.6799	2.2072	2.8016	1.0855	1.1217	49.17995651
Sausage 50% SMB 50% SPI	1.2503	2.0608	2.2971	1.0140	1.0468	49.20419255
Mayonnaise EWP	1.8811	2.8844	3.8180	0.9475	1.9369	32.8491194
Mayonnaise EWP	1.8811	2.8844	2.8682	1.8973	0.9871	65.77797809
Mayonnaise shrimp	1.1982	2.2693	2.7155	0.7520	1.5173	33.13797206
Mayonnaise shrimp	1.0226	2.2015	2.4765	0.7476	1.4539	33.95866455
Mayonnaise cod	0.9338	2.2333	2.4889	0.6782	1.5551	30.36761743
Mayonnaise cod	1.0465	2.2397	2.6057	0.6805	1.5592	30.38353351

Table 32 - Raw data for the Lowry protein determination.

Dilution	Sample	Absorbance	Protein (g)	Protein (%)
8000	Fish ball control	0.0172	3.675	2.94
8000	Fish ball control	0.016	3.375	2.7
8000	Fish ball control	0.0148	3.075	2.46
27000	Fish ball 100% SPI	0.0134	2.725	7.3575
27000	Fish ball 100% SPI	0.0111	2.15	5.805
27000	Fish ball 100% SPI	0.0076	1.275	3.4425
16000	Fish ball 50% SMB 50% SPI	0.0393	9.2	14.72
16000	Fish ball 50% SMB 50% SPI	0.0352	8.175	13.08
16000	Fish ball 50% SMB 50% SPI	0.0348	8.075	12.92
8000	Fish sausage control	0.1274	31.225	24.98
8000	Fish sausage control	0.1245	30.5	24.4
8000	Fish sausage control	0.1413	34.7	27.76
16000	Fish sausage 50% SMB 50% SPI	0.0442	10.425	16.68
16000	Fish sausage 50% SMB 50% SPI	0.0462	10.925	17.48
16000	Fish sausage 50% SMB 50% SPI	0.046	10.875	17.4
1000	Mayonnaise shrimp	0.1202	29.425	2.9425
1000	Mayonnaise shrimp	0.1211	29.65	2.965
1000	Mayonnaise shrimp	0.1176	28.775	2.8775
1000	Mayonnaise cod	0.0513	12.2	1.22
1000	Mayonnaise cod	0.0608	14.575	1.4575
1000	Mayonnaise cod	0.0521	12.4	1.24
1000	Mayonnaise egg	0.098	23.875	2.3875
1000	Mayonnaise egg	0.1281	31.4	3.14
1000	Mayonnaise egg	0.1362	33.425	3.3425
1000	Soup shrimp	0.0136	2.775	0.2775
1000	Soup shrimp	0.012	2.375	0.2375
1000	Soup shrimp	0.0112	2.175	0.2175
100	Soup salmon	0.5842	145.425	1.45425
100	Soup salmon	0.5617	139.8	1.398
100	Soup salmon	0.5639	140.35	1.4035
1000	Soup control	0.0832	20.175	2.0175
1000	Soup control	0.1004	24.475	2.4475
1000	Soup control	0.0634	15.225	1.5225