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# **CIRCULAR APPROACH IN FISH INDUSTRIES**

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# ABSTRACT

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The growing population requires a lot of food in order to sustain itself. Therefore, also, more fish need to be caught and farmed. Since a large amount of fish captured cannot be used commercially, it is usually thrown away, which in turn can be hazardous to the environment not to mention sea birds. Bycatch is also caught when fishing. A circular approach to this fish waste means seeing this waste as something that should be used instead of being thrown away.

Usually fish waste if not discharged overboard, is converted into fish meal or fish oil. It is often costly and time-consuming. The heterogeneous nature of fish waste is an added challenge because finding solutions that work all the time with all types of fish waste is not possible.

The lab-scale applications mentioned in this thesis include, but are not limited to, turning fish waste into fertilizer, feed, nutrients, peptides, enzymes, hydrolysates, biomaterials, wastewater treatment products, and biofuels. This thesis was a literature review, and no lab work was done when making it. Fertilizer and feed are relatively straight-forward to do, but they require more time to be aged than their commercial counterparts. Nutrients, peptides, hydrolysates, and enzymes can also be extracted from the fish waste; however, in the case of mincing the fish waste it can only replace part of the fish meat.

Peptides showed interesting properties but required more research to be identified precisely. Hydrolysates could be used for capturing moisture in the food industry, but their inner bitterness needs to be masked beforehand. Enzymes can be extracted, but their performance varies; an enzyme for dehairing leather works great, but an enzyme to make cheese creates a cheese that cannot hold moisture inside it. Fish waste can also be added into packaging materials, where the biodegradability increase, but this comes at the cost of weaker strength and an inability to hold moisture inside the package. Their properties can be improved by adding additional substances into the material.

Fish waste can also be used to manufacture sorbents for treating wastewater and in the articles they could remove, for example, azo dyes and lead. Fish waste can also be converted into biofuels. However, they increase some emissions, while simultaneously reducing others and they cannot be stored as long as conventionally made diesel. Fish waste has many potential applications, but their large-scale implementation requires massive investments in a circular economy.

Key words

circular economy, fish, fish industry, fish waste, fish waste applications

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### **1 INTRODUCTION**

The ever-growing population requires a lot of food in order to sustain itself. Since the growth in processing fish comes solely from aquaculture, what follows is the growing amount of fish waste not to mention the waste being thrown overboard all the time from the fishing vessels. The circular approach refers to using as much as possible and throwing away as little as possible. To apply it to the fish industry is to use these waste streams and turn them into something useful instead of discarding them. This thesis aims to introduce the fishing industry, showcase different types of fish waste, its volume, and its composition. Then different methods that could be used to analyze the fish waste and its treatment techniques are covered. The most significant part of this thesis covers possible lab-scale applications that were summarized from scientific articles. The thesis ends with a conclusion that provides insight on the prospects of circular economy in the fish industry.

This thesis is a literature review, and no lab work was done while making it. It covers only fish waste generated in the industry, and therefore it does not cover other fish waste streams such as fish being thrown away in shops. Due to a lack of research, possible applications of fish waste in Finland could not be found, and therefore, the applications here are collected from resources abroad.

### **2 FISHING INDUSTRY**

Fish production globally was roughly 171 million tons in 2016, with a value estimated at USD 362 billion, with the majority originating from aquaculture. The production of fish by capturing them has stagnated since the late 1980s, and the growth nowadays comes from aquaculture only. The top producer of fish in 2016 was China, and the Alaska pollock was the most popular fish species, surpassing runner-up Anchoveta and Skipjack tuna. Northwest Pacific remains as the number one spot of landing fish. However, it is estimated that 27 percent of the landed fish is lost before it even reaches the end consumer. (FAO 2018.)

The fish consumed in Finland mostly comes from abroad, and around half of this fish originates from Norway, where it is farmed in aquaculture. The most significant fish used commercially is the Norwegian salmon, whereas, in the past, it used to be the Baltic herring. More than half of the Baltic herring is used as part of the feed for fur animals. (LUKE)

The fish landed keeps on decreasing all the time, so aquaculture and importing fish come into question to ensure the current production level. Production costs also rise due to expensive fuel used to operate the fishing vessels. The main three branches of the fish industry are fish trade, canning, and smoking industry. Also, aquaculture requires fish meal from the landed fish. Even the fish by-products are divided according to the demands of the recipient; there is large-scale collection to produce fertilizers, energy and livestock feed, and small-scale collection of fish waste for the pharmaceutical industry. The main differences are in the expected quality that must be high due to regulations in the small-scale collection, where the fish needs to be cooled down fast and the necessity of tracking them back into the place where they were captured. (Penven, A., Galvez, R. & Berge J.-P, 2013) Marine fish is usually classified either as lean (with less than 3% fat in the fillets), medium (3-7 %), or high fat (more than 7%). (Clark, Jung & Lamsal 2014, 531.)

Fishing practice can be divided according to fishing methods and fishing gear, both of which have changed considerably over time. In the case of fishing gear, various nets can be used to catch fish. The function principle of a net is simple: the top part of the net is floating due to, for example, corks, and the bottom part of it is weighted. Once fish are lured in, a purse line that encircles the opening of bottom net is used to close the net. (Granata, Flick, & Martin 2012 15.) The final products of the fishing industry can be divided into ready-to-eat, ready-to-cook, frozen, and processed types.

The purpose of all fish processing is to prevent the bacteria and enzymes within the fish from degrading it after catching it. This prolongs the shelf life of the catch and ensures that the fish is safe to consume. The processing affects only the microbiological properties of the catch and leaves other properties like biotoxins unaffected. The fish can be categorized in different ways depending on how it will be prepared before consumption, with the fish species that will be consumed without cooking them first posing the highest risk and the ones that are dried before consumption having the lowest risk. Also, great care must be used to ensure that regardless of the environment where the fish is being grown, it should enjoy stress-free conditions. The processing methods use various ways to treat caught fish, for example, chilling, packing in a protective atmosphere, heating, and irradiation with ionizing radiation. (Boziaris 2014, 1-5.)

Fish processing plants usually have the following steps in turning caught fish into sale-ready fish. First, the fish are stunned to prevent any unwanted changes in the structure of fish. Then the fish are separated according to their species and size i.e., they are graded. Then the slime on top of the fish is removed to prevent the fish from spoiling. Next, the fish are scaled to get rid of any unwanted pathogens that are growing in them and to help in keeping the fish fresh. What follows is then the washing of the fish with pressurized water; if the fish caught was from freshwater, the water used to wash them should be potable. Next, the head of the fish is removed, it is gutted, and the fins are cut. (Ghaly, Ramakrishnan, Brooks, Budge & Dave 2013.) Fish fillets can be prepared bone-in, boneless, or skinless. Not all meat from the fish can be secured in the form of a fillet, but rather it continues its journey as mince. Mince is the flesh that is separated from the bone, and it is usually frozen for later use and added later into other products. The quality of fish mince is determined by the quality of raw material and the separation process. (Granata et al. 2012 105-106.)

One of the ways to produce mince is the so-called belt-and-drum system. Here, headless and gutted fish is crushed between a crusher roll and a perforated drum. Fish mince continues forward through the holes of the drum, and a tension belt moves the waste away from the process. The most significant parameter that influences the quality of mince is the size of the perforation; for example, smaller perforation lowers the amount of bone that ends in the final product, but it also damages the texture of the fish mince. In general, 3-mm openings are used to get as little bone as possible with an acceptable mince texture. After separation, the mince is washed and stabilized. (Granata et al. 2012 106-110.) The production of fillets requires a steady stream of fish that is packed into boxes with ice onboard the ship. The boxes are stored in a storage room with a temperature of  $2^{\circ}$  C, and before treatment, they are deiced, processed, and packed in polystyrene boxes. These boxes wait in another storage room for transportation. Smaller fish

such as herring are pumped into the tank onboard, and after draining the chilled water, the fish is conveyed further and processed directly. (Boziaris 2014, 44-45.)

Aquaculture refers to the controlled cultivation of fish in a pond. Usually, there are three different types of tanks in a fish farm: the hatchery, and two other tanks. In the first one the fish are weaned under a rood, and when they reach a specific size, they are moved into another tank. This last tank is the one where the fish will be kept until they have reached the size when they can be sold, and this tank can also be built without a roof above it. The fish can also be divided into a monoculture, where only one fish species is grown or a polyculture where the fish species are paired with another species like rice. The feed is used to encourage the growth of fish to be normal, but it can also be wastewater. The types of aquaculture vary depending on the amount of technology and, therefore, the money invested. The more intensive the aquaculture gets, so does the risk of illnesses spreading on the farm. This is because fish are under constant stress to ensure their best performance. The water can stream through the aquaculture once, or it can be used in the system several times, provided that it is treated accordingly. If pumps are used to move the water around the system, control of the level of oxygen is necessary so that it stays stable. This treatment refers to filtering the water before letting it reach the fish to prevent too high concentrations of certain nutrients. The water can also be disinfected, heated, cooled, and oxygen may be added to improve the water quality. (Lekang 2013, 1-4.)

In waste-fed aquaculture fish in the pond are integrated into the wastewater treatment by letting them feast on the nutrients in the wastewater. This helps the wastewater treatment further downstream by removing some of the organic nutrients. However, it does very little in removing, for example, the pathogens and heavy metals from the wastewater. It is an interesting concept, but it requires more research on how to ensure safe feed for aquaculture. (Polprasert & Koottatep 2017, 328-334.)

#### **3 FISH WASTE**

Fish waste refers to the part of fish that is left over after for example fileting a fish, such as skin and bones. Also, when fishing, some other species can be caught by accident which are then discarded; this is called bycatch. The fish waste can be treated onboard the vessel or on solid land, when the vessel arrives at the harbor. Edible products of fish waste include, among others, mince, fins, and roe. Fish mince is susceptible to degradation and color change. The first problem can be treated with physical processes and latter with chemical processes. Protein powder is another option for sale from the raw materials. (Granata, et al. 2012 106-110.) Due to the high commercial value of roe, it is always collected from the fish and sold further. Fish heads have relatively little meat in them, but the tongues and cheeks can be sold further. For example, one can recover Omega-3 fatty acids from the livers of lean whitefish that originate from cold waters. Most fish waste is either burned, dumped into the sea, or disposed in landfills. (Saranya, Prasanna, Jayapriya, Aravindhan, & Tamil 2016.)

The remaining waste is converted into fish meal and oil. Fish meal is used to feed among others fish, shrimp, and livestock. (Granata et al. 2012 130-131.) Other uses are covered in chapter 4. The disposal of fish waste can create a long-lasting impact on the environment after dumbing the fish waste back into the ocean. (Cadavid-Rodriguez, Vargas-Munoz & Placido 2019.)

One challenge in fishing is not only the bycatch of fish that is not wanted but also other creatures, such as sea birds. The reduction of this bycatch was the goal of one research group in New Zealand by mincing fish waste or processing waste into fish meal before dumping it into the ocean. The accidental capture of sea avians has been connected to the decline in their population. Birds can, for example, get captured by the fishing net hosting their prey. To avoid this, the fish waste can either be minced before discharge or kept in the ship and turned into fish meal (Abraham, Pierre, Middleton, Cleal, Walker, & Waugh 2008.)

#### 3.1 Fish waste composition & analysis methods

The by-products of the fish industry include, for example, proteins, minerals, and fats. (Penarubia 2017). Majority of the fish is moisture, a small part is protein, and the rest is fat (Ghaly, Ramakrishnan, Brooks, Budge & Dave, 2013). Fish waste could be analyzed with the same methods as fish in the industry. Since the material is waste, it usually ends up in the landfill, burned, or dumped back into the sea. When the material is dumped into the sea, the bacteria require oxygen to work on it. As a result, the fish waste depletes the amount of oxygen in that part of the ocean, where the waste was dumped. The other substances, such as ammonia, alter the pH in the area and decompose algae. (Ghaly, Ramakrishnan, Brooks, Budge & Dave 2013.)

All research articles use different methods according to the goals of the project. The most popular methods for quality control in the fishing industry are near-infrared spectroscopy (NIR), imaging spectroscopy, NMR spectroscopy, and X-Ray imaging. These are the methods of choice because they work quickly, and they do not destroy the sample fish. NIR spectroscopy relies on emitting light on the wavelength a little below the range of visible light. It provides a rapid method to assess and determine: for example, the fat content of the fish. However, the instruments working in this way cost a lot of money and require large-scale data collection to prove any correlation. Imaging spectroscopy is a novel method where the shape of the sample is showcased with composition analysis as a bonus. This method is based on the interaction with light and the sample. The most significant benefit of this technique is the ability to locate possible deviances in the samples in addition to detecting them. NMR uses electromagnetic radiation and the properties of atoms to provide information about the substance. In research, it has been used to quantify the number of fatty acids, for example, the content of polyunsaturated fatty acids and whether they are saturated or unsaturated. But in the fishing industry, largescale implementation is still inhibited by the high price of the technology. X-ray imaging emits X-rays to find density in the sample in question. For example, it could be used to detect bone fragments in fish. All of the before mentioned methods are promising in the lab scale. However, their cost and the sporadic samples provided by heterogenic material make it difficult to find an analysis method that could work in all applications. (Nollet & Toldra 2010, 121-134.)

#### 3.2 Volumes of fish waste

More than half of the fish caught is not used in the industry and ends up being discarded (Arvanitoyannis & Kassaveti 2008). Other sources mention that 35 % of the catch is lost (FAO). In Columbia, roughly 45 % of the catch goes to waste (Cadavid-Rodriguez, Vargas-Munoz & Placido 2019.). Another source quotes that only 40 % of the catch is converted into an edible form, and the rest gets discarded (Dekkers, Raghavan, Kristinsson & Marshall 2011 [Ockermann & Hansen, 2000, Raa & Gildberg, 1982). The amount of waste generated increases the more processed the fish is and the size of the industry also influences: larger plants also generate more waste. (Galvez, Carpio, Moreno, Guadix & Guadix 2013).

#### 3.3 Types of fish waste

Since the type of fish waste is always different due to by-catch, region, time, and other variables, there is no standard fish waste. However, it can be presumed that fish waste has similar characteristics to fish itself. In an average fish fillet, about 70 to 80 % is water, and this water is tightly connected with the proteins in the fish. The proteins are divided into three groups: sarcoplasmic, myofibrillar, and stroma proteins. The minor part of the fish muscle consists of sarcoplasmic proteins. It also stores the enzymes in the fish that rot the fish if left to its own devices. The major part of the fish muscle is made from myofibrillar proteins; here, the flesh is even more sensitive to changes in the environment than the previous example. When these two proteins are removed, the part left is referred to as stroma proteins. The amount in muscle can differ from 3 to 10 %. It houses the binding elements such as gelatin, and the changes in fish quality are usually caused by changes in the collagen structure. The rest of the fish is non-protein nitrogen compounds, lipids, carbohydrates, minerals like calcium and vitamins like vitamin A. (Gokoglu, Yerlikaya & Geokoglu 2015.) Fishbones contain, among other substances calcium (Ki-zilkaya & Tekinay 2014).

#### 3.4 Fish waste treatment

The most significant methods for fish waste treatment are hydrolysis, bioremediation, and anaerobic treatment, with the addition of mechanical processes such as filtration and screening. Hydrolysis refers to a process where fish biomass is converted into edible protein products. Bioremediation means converting a habitat from a polluted environment back into its natural state with the help of biofilters. Fish waste can be used in the production of the biofilter. (Boziaris 2014, 265-270.)

Fish meal is made similarly to fish oil by heating fish waste, decanting the oil, and then capturing the solids by, for example, centrifuging them. If the waste is hydrolyzed with the help of enzymes, a large selection of functional quality proteins are produced. One example of such a process is to add fish viscera into the slurry. These hydrolysates have hygroscopic properties, and therefore they can be used to absorb air moisture. (Clark, Jung & Lamsal 2014,506-507.) One option to use is to turn the fish into compost; it has high nitrogen content and the ability to release it slowly over time. It is manufactured by combining standard green and wood waste with the fish waste to absorb the excess moisture. The main drawback is that it requires much time to age; otherwise, the result is too oily. (Emerson, 2013.)

Alternative option for turning fish waste into fish mince was evaluated in the scientific articles. The various options of doing this are either mechanical or boiling technique, due to the potential to generate revenue from waste and to prevent dumping the fish waste directly into the water. The first method was to remove the fillets from the fish, then the head, tail, and dorsal fins and after the housing rib was cut away. After that, the fish meat was boiled for five minutes to remove any fishbone left. The mechanical method was to place the fish without fillet, head, fins, and viscera into a machine, where the fish was deboned. After this, the minced fish was washed in a saline solution and pressed to remove excess salt and water. With the boiling technique, 42 % of the waste was converted into fish meal, and with the mechanical process, 54 % was acquired, and because of these numbers, it was recommended to utilize the mechanical alternative for the production of fish mince from fish waste. (Leira, Nascimento, Alves, Orfao, Lacerda, Botelho, Reghim & Lago 2019.) Another alternative researched in Indonesia is to utilize the phosphorus-rich waste generated in aquaculture and use it as a fertilizer to grow soybeans in peatlands. Since peatlands require large amounts of fertilizer to sustain the production of soybean, fish pond sludge waste provides a way to solve the waste problem and return the nutrients to the environment. After various tests, the amount of inorganic phosphorus fertilizer could be cut in half when 220.78 grams of sludge was introduced into a bag of 50 x 50 cm dimensions. Further research needs to be done in order to study what residues are left from using this type of fertilizer. (Erina, Nyahu, Sih, & Soaloon 2018.)

One option to treat effluent water from fish farms is a mechanical treatment to remove suspended solids from the wastewater. The research aimed to provide methods for removing pollutants from the effluent of an intensive fish farm. The tests were run when the plant was polluting the most at its production peak. The samples were collected, analyzed for the number of nutrients, total number of suspended solids and fecal particulate matter. Sedimentation trials were carried out. The effluent was rich in nutrients due to massive amounts of feed used in the fish farm. However, pollutants at this level are only available four months per year. The researchers suggested a filtration system with a 50-micrometer mesh to remove solids, which could be ineffective if the number of solid particles that are larger in diameter then the diameter of the openings in the filter is much. The filtration system takes less space than the sedimentation channel that the farm used, and it removes suspended solids better. (Bianchi, Centoducati,nToteda & Onofrio 2011.) Fish oil is the part left over after cooking fish remnants. The product then goes through decantation, centrifugation, and refining. However, some ships choose to burn the fish oil in the engines of the ship, because it is more convenient than taking it to the harbor. (Clark, Jung & Lamsal 2014, 531.)

### **4 FISH WASTE APPLICATIONS**

#### 4.1 Fertilizer and Feed

One option to treat fish waste is to ferment it so that it is converted into feed for livestock. Turning fish waste into fish meal is quite expensive, and that is why researchers have researched about fermentation as an alternative. The fish waste used here was viscera of the carps, where the guts were removed using clean water. Fresh whey was collected from the local daily production so that it could provide the lactic acid bacteria for the fermentation. Also, cane molasses were added to provide a source of carbohydrates in the fermentation. After various tests, it was discovered that the whey from the dairy industry is an excellent source of lactic acid bacteria, and therefore, it can be used in fermentation. The quality of the feed supplement produced was sufficiently good, and only a little crude protein and lipids were lost in the process. Other properties included the fact that it was microbiologically safe, and the nutrients would suffice in the intended purpose. (Samaddar & Kaviraj 2014.)

One option to treat fish waste is to turn it into silage and feed the newly-made product back into the process. Fish silage means a mixture of fish pieces and a preservative to stabilize the mixture. Due to the nature of the liquid solution, proteins are pre-digested, but the nutrients are similar to regular fish meal. The fresh and uncooked fish parts are combined with an acid to create a digestive system, where the proteins are disintegrated into peptides and amino acids. The finished product can be fed, for example, to livestock or used as a fertilizer since the proteins are dissolved in the process and create nitrogen and originating from bones of the fish waste some minerals are included in the fertilizer. The peptides work against bacteria growth. (Penarubia, 2017.)

Another research mentioned that one option to treat fish waste is to turn it into fertilizer in liquid form. The option to convert it into fish meal requires a lot of energy. The fish waste does not contain common carcinogenic substances like other effluents from industrial or domestic processes. The feasibility of turning waste into compost was determined in a reactor. The bacteria to degrade fish waste can be recovered from the viscera of earthworms. The fish was autoclaved i.e., cleaned with steam and fish oil was removed. Then the bacteria, sterile fish waste, and distilled water were added. Then air was introduced in order to encourage the growth of bacteria. Using an autoclave was necessary to ensure a faster reaction rate. With the addition of lactate to prevent decay, the liquid fertilizer was on par with commercial equivalents. (Kim 2011.)

One option is to combine seaweed and fish waste and turn them into compost for use in organic agriculture; however, before application, the nutrients in the fertilizer need to be mineralized to ensure a consistent flow of nutrients that the plants use. Experiments were conducted with a compost, which had a mixture of seaweed, fish waste, and pine bark chips, and which was produced in 10 weeks. Tests were run on different rates of fertilizer, and the mineralization of carbon and nitrogen were noted at regular intervals. The carbon mineralization made the compost stable, and the nitrogen mineralization was sufficient to replace commercial alternatives. (Vives-Illera, Lopez-Fabal, Lopez-Mosquera & Ribeiro 2015.)

One research group in Poland evaluated how including fish waste in the compost could improve crop yield and what elements would accumulate. In the experiment, sawdust was used to build up the compost, pine bark for the same reason; some had lignite in them, wheat straw, and small fish. All the components were ground, placed in layers so that the organic layer was on top of the compost, and different compositions of compost were researched. The highest yield was reached by using a compost that had fish waste, straw, lignite, and bark. If mineral nitrogen ready-to-be-used by the plants was available, the content of zinc and copper decreased. Further research is needed to study this phenomenon. (Radziemska & Mazur 2015.)

## 4.2 Enzymes & Hydrolysates

Hydrolysates that were extracted from fish waste which have specific peptides contain some that stimulate growth. These could be fed to aquaculture. However, the change observed in the experiments was not significantly different from the control group. Some can enhance enzymes being emitted into the gastric system, but no clear correlations could be established. Some of the peptides can boost the immune system of the fish, specifically the part that is non-specific, and the peptides could be added directly into the feed to boost the immune system of the fish. Other peptides can regulate appetite and have great potential in the development of weight control medication. The peptides that are manufactured are dependent on the fish waste type, species, enzymes, and reaction parameters of the hydrolysis. However, more studies are required to understand the behavior of these hydrolysates fully. (Ravallec, Cudennec & Dhulster 2013.)

Fish protein hydrolysates have many interesting properties that can be beneficial in future applications. The properties were created in the enzymatic hydrolysis that created the products. One of these features is high solubility, followed by emulsifying properties, that are due to their hydrophilic and hydrophobic group in the hydrolysates. Then, there is the capacity of fish protein hydrolysates to absorb fat, which varies according to the species. Like emulsification, the foaming properties of the hydrolysates can be useful in the food industry. The foaming can be influenced by altering the pH, which reduces the foaming or by altering the hydrolysate concentration. The ability of the fish protein hydrolysates to absorb water is also of great importance to the food industry, and this ability can be influenced by the enzyme that is used in the manufacturing process. However, the greatest challenge has proven to be the bitterness of the hydrolysates, which can be combatted by removing it by activated carbon or by masking it with specific amino acids. (Pires & Batista 2013.)

Fish protein hydrolysates can be manufactured from fish waste, and these are of great interest since these substances are disintegrated from larger amino acids, and their properties can be applied in the food industry. The process was called enzymatic hydrolysis. Here, the fish waste was minced and mixed with water so that for every part of fish, there were two parts of water, and the mixture was blended and homogenized. Then the papain enzyme was added, and the reaction happened at 60 degrees for three hours. After stopping the activity of the enzyme by increasing temperature, the product was filtered, and the liquid phase was separated for 24 hours to remove fat. After running a few tests, it was concluded that fish waste could be used to produce protein hydrolysates. (Saputra & Nurhayati 2016.)

A research group envisioned a research method for extracting proteases from the viscera of a fish, pirarucu. It was selected because of its size which also meant large amounts of fish waste. The fish were fed a commercial diet for 40 days, and after that, they were dissected. A specific part of the fish called pyloric caeca was removed, cleaned with deionized water, and transported at 4 degrees into the laboratory. The tissues were then homogenized, centrifuged, and then the enzyme was purified. First, the tissue was treated with heat, then precipitated with ammonium sulfate and in the placed in affinity and affinity chromatography. The challenge preventing the large-scale utilization of fish waste for the production of enzymes is the prohibitive cost of the protein purification. Inhibitors and specific substrates were used to identify that enzyme in question was a protease called trypsin. The enzyme showed great stability over a large alkaline pH range, while also being thermostable and therefore holding great promise for further industrial applications. (Freitas-Junior, Costa, Icimoto, Hirata I, Marcondes. Carvalho, Oliveira & Bezerra 2012.) A research group tried to isolate a lipase from fish processing waste. Because lipases catalyze a wide range of chemical processes, the one in this experiment *Enterococcus durans* is usually used in improving the flavor of cheeses. The enzyme was extracted from fish processing waste, identified and incubated for 3 to 7 days, and after fluorescent light was seen in UV light, the presence of the lipase could be confirmed. Normally carbon and nitrogen are provided into the growth system with dextrose and some extracts. However, in this study, fish waste oil replaced carbon, and fish waste protein hydrolysates replaced the nitrogen. The extracted enzyme could be of interest to the food industry. (Ramakrishnan, Goveas, Halami & Narayan 2015.)

Another research group tried to use enzymes from tuna waste and check their applicability for making cheese. The enzyme in question was found in the stomach of the fish, which were then acquired for testing. The fish were caught and their stomachs were removed, frozen and later thawed, split, cleaned and rinsed three times in tap water. Specifically, the mucosa lining was cut from the stomachs, chopped into tiny pieces, and homogenized in distilled water and sodium chloride. The next day the mixture was converted into a slurry, which was then centrifuged, and then the gastric proteases were extracted. The enzyme required a specific temperature and pH to be activated. However, the cheese made by enzymes from fish waste was less desirable, than cheese made by standard enzymes. In addition, the tuna enzyme cheese produced drops of moisture on top of the cheese, indicating poor ability to hold moisture. Further research needs to be carried out in order to turn this cheese enzyme into something commercially feasible. (Tavares, Baptista & Marcone 1997.)

Another option is to create enzymes from the fish waste and use them in the leather industry. The method of turning fish waste into fish meal requires a lot of energy, and therefore it is not always feasible. The dehairing of leather is normally carried out with lime and sulfide generates, with the tanners having the added option of using commercial dehairing enzymes. These are not preferred due to their high cost, which comes from the fact that additional purification steps are required since the enzymes have a microbial origin. Also, the pollution load of using lime and sulfide generates is massive. The protease enzyme was extracted from fish waste, which was rinsed with distilled water, thawed at room temperature, screened, precipitated, centrifuged and dissolved in a buffer. After conducting experiments with conventional control substances and these new enzymes, it was discovered that the enzymes removed all hair and they did this with the same performance as their commercial alternatives, while simultaneously emitting a smaller pollutant load. (Saranya et al. 2016.)

One research tried to discover if it is possible to produce industrial enzymes from fish waste, proteases to be specific. First, the fish waste was rinsed with distilled water, thawed at room temperature for two

hours, cut into small pieces, homogenized with HCl buffer, filtered, and clarified by centrifuging. Ammonium sulfate was used to extract the protease, which was then analyzed in different pH ranges, temperatures, and in the presence of metal ions. The tests proved that a protease was created and that the optimal pH range for this enzyme was from 8 to 10. It worked in temperatures that are typical for industrial processes, and with certain organic solvents, such as ethanol, the performance improved, and they also improved the stability of the enzyme. Some metal ions such as aluminum could activate the enzyme whereas others like potassium inhibited it. (Saranya, Jayapriya, & Samil 2018.)

#### 4.3 Biomaterials and biofillers

A research team tried to produce HAP (hydroxyapatite) powder from fish waste so that it can be utilized as a biomaterial. Here the fish waste was washed only with tap water for one day and with a sodium hydroxide solution the day after. Sodium hydroxide was added to remove the proteins from the fish. Then the fish was washed, this time with distilled water, and they were dried and treated with hydrogen peroxide to bleach the fish, which was in turn washed away three times with distilled water. The treated fish bones were calcinated at 800 Celsius degrees for five hours, and in the end, HAP was created. The presence of the HAP in the lab rats caused inflammation. However, it ceased almost entirely after 30 days. It could be used for example in dental applications, but it shows mild carcinogenic properties. Further studies are required, especially regarding its biocompatibility. (Yamamura, Pereira da Silva, Ruiz, Ussui, Lazar, Renno, & Ribeiro 2018.)

A research group tried to solve the problem of off-shore dumping of fish waste by trying to incorporate fish waste in different amounts in foam packaging material. The fish waste was ground two times, then collected on a pan with porcelain coating, turned into a kind of paste at a temperature of 60 degrees for 16 hours, and finally ground until it was fish powder. The fiber used was recycled, and it was dispersed into water by stirring it for 15 minutes. Then the fibers were combined with pre-gelatinized starch, magnesium stearate, and fish powder. These were mixed for about 5 minutes until the desired consistency was reached. The mixture was placed in the center of the mold, that was pre-warmed to 190 to 200 degrees. Magnesium stearate was used as a filler and to prevent the mixture from becoming too sticky. Also, Starch-Polyvinyl Alcohol (PVOH) films were made by mixing starch, polyvinyl alcohol, fish powder, glycerol, and deionized water. The cast films were manufactured by pouring the mixture onto a glass plate so that it was covered entirely, and then it was left there to dry. The materials were tested and found out to have comparable properties with typical commercial films, such as poly-

styrene. They degraded fast in compost, but their resistance against moisture was lacking, and therefore, they need more substances added into them to make commercial applications feasible. (Imam, Chiou, Woods, Shey, Glenn, Orts, Narayan, Avena-Bustillos, McHugh, Pantoja & Bechtel 2008.)

One research focused exclusively on the challenges faced by people trying to extract collagen from fish waste. Collagen is usually extracted, for example, from fish waste due to the risk of hazardous prions from cattle. To work in the process, the fish waste needs to be demineralized. However, some researchers chose not to do this. Also, purifying the collagen is critical to prevent any changes in the material by non-collagen proteins. Using sulfuric acid to control the swelling of the skin can lead to the removal of the collagen from the fish waste. The collagens need to be extracted at 4 Celsius degrees rather than at 25 to 35 degrees because, in higher temperatures, the extracted material becomes gelatin. The extraction should happen in acidic conditions. When these factors are considered, the maximum recovery of collagen can be ensured. (Kumar & Rani 2017.)

An alternative to using fish waste is to employ fish scales as a component in composite materials. Composite materials are substances that have several different components. However, not all of them are degradable. This problem could be fixed with new biofillers, i.e., a filler from the nature that could replace some of the material in the composite. Here the researchers washed, dried, and boiled the fish waste in water with a small amount of acetic acid to create a viscous mixture. Then samples were prepared using the same amount of epoxy resin and curing agent, but with different amounts of fish scales. After various test methods, it was discovered that the addition of fish scales decreased the tensile strength of the composite. It increased the plastic behavior (object returns to its starting position) and enhanced their ability to receive impact. The materials also became more biodegradable, since the organic nature of the fish scales possessed lower stability in higher temperatures. (Razi, Islam & Parimalam 2019.)

Another application of fish scale waste is to apply them as a filler in starch foam. Starch foam is an environmentally-friendly alternative for the polystyrene used in takeaway foam boxes. The most prevalent method is to bake them; here, the starch is gelatinized, and the water within is vaporized. However, it has poor mechanical and thermal properties, so additional additives are needed. Because fish scale waste has a large amount of calcium, it can be used to improve the characteristics of the composite. To start the experiments, the fish scales were washed, dried, ground, and dried again to prepare them for the experiment. Then the powder was calcined in two different ways, one at a high temperature for a short amount of time and the other one at a lower temperature for a longer time. Calcination removes some organic compounds from the fish scale powder, which, if not removed, will lower the adhesion of the material i.e., it cannot stand such large impacts. Starch foam with fish scale powder resists water better, and it has higher thermal stability due to the inorganic substances in the starting material. (Chiarathanakrit, Riyajan & Kaewtatip 2018.)

Another application of fish waste is to extract the gelatin from fish waste and use it in packaging. Fish gelatin is a protein commonly found in fish bones and skin. However, the characteristics of pure fish gelatin are not commercially feasible, because it is not strong enough, has trouble to stay stable in humid conditions and holding water inside the package. However, it can prevent the oxygen from entering the package, and therefore the only question remaining is how to combine something with the gelatin film to improve its properties. Fish gelatin film is manufactured by hydrating the fish gelatin in distilled water, stirred, and a plasticizer is added. The mixture is poured on a flat surface to allow the solvent to vaporize; the film is created when the proteins connect. The plasticizer used was glycerol, and it was added to improve the properties of the film; to reduce its brittleness, for example. Other materials can be blended into the film, such as Chitosan, a cationic polysaccharide. In the right pH range, it can enhance the properties of the packaging film by improving its water stability. To further lessen their rigid nature, fatty acids could be introduced into the film, but this choice is problematic due to the high costs of the fatty acids. Another choice is to add essential oils into the mixture, but the effects are dependent on the specific oil. Some increase the flexibility, but they make the film more fragile at the same time, as is the case with basil essential oil, whereas, for example, using essential oregano oil lowers the strength of the film. (Hosseini & Gomez-Guillen 2018.)

One other option is to use cross-linking agents to connect the polymer chains in the film. Using chemical cross-linking is usually avoided due to the unwanted effect of toxicity. That is why organic acids are preferred, for example, citric acid. It can act as a plasticizer, and if some of the citric acids are not consumed in the reaction, it does not pose a problem because of its non-toxic nature. Enzymes remain an option to cross-link polymers, but their high price prevents large-scale applications. In physical processes where: for example, lactose is added, or the film is heated, changes are not significant enough to make any difference. One way to improve the performance of these films is to add nanosized fillers in them, for example, silica nanoparticles. (Hosseini & Gomez-Guillen 2018.)

One material highlighted by the research team is the layered silicate nano clay which when included in the film, can prevent the packaged material from interacting with the environment. Also, nanowhiskers and fibers that originate from biomass such as cellulose can be used. As an example, cellulose nanofiber was highlighted in the article. Cellulose nanofiber can be extracted from timber with chemicals, enzymes, or with tools. A network of these nanofibers could be used to reinforce the biocomposites. One real-world application is to use silver nanoparticles in food packaging, due to their tendency to decimate bacteria. The work is far from done since more research is required to make sure that no nanoparticles move from the packaging into the food. (Hosseini & Gomez-Guillen 2018.)

Because of such concerns, fish gelatin has been considered as packaging material, since it has already the potential to thwart bacteria. In some instances, the best outcome was achieved by combining essential oil and packaging made from fish gelatin to create a new material. Antioxidant food packing is the other option, where fish gelatin packing could be combined with green tea powder in order to create a packaging that protects the food from oxidation. Fish gelatin holds a lot of promise in becoming the packing material of tomorrow, but right now, it cannot compete with normal plastic. (Hosseini & Gomez-Guillen 2018.)

#### 4.4 Waste treatment

One option in creating substances for waste treatment from fish waste is to pretreat fish waste bones and use them as sorbents to remove heavy metals. The composition of the bones is mostly inorganic hydroxyapatite that can remove, for example, lead. The bones were extracted by removing them from the fishes, and they were then washed with distilled water a few times. When the cleaning process was complete, the fish bones were dried in an oven and later mortared to the micrometer range. After analysis, the best removal rate was obtained at 50 degrees. When the pH was increased, so did the adsorption. Leaching or desorption of lead back into the wastewater was minimal, so the research team recommended this waste treatment method of using sorbents made from fish waste bones. (Kizilkaya & Tekinay 2014.)

One other application for fish waste is to use it to prepare highly graphitized and heteroatom doped carbon to remove azo dyes from wastewater. The production of doped carbon is costly, and therefore, using fish waste to manufacture it becomes an alternative. Nitrogen can be used to dope the carbon, which in turn is readily available in fish waste. The fish waste was washed with de-ionized water and dried after the process in an oven for one day. Then it was pre-carbonized for one hour in 300 degrees nitrogen-rich conditions. Then it was mixed with potassium hydroxide and activated in a nitrogen-rich environment for one and a half hours at 700 degrees. The final product was obtained after washing the remnant dark solid with HCl and after that, with de-ionized water, and at last, dried in an oven for one day. The idea in adding KOH was to create enough pores, and heteroatom rich fish waste created

enough active sites. These proved to be successful in creating a substance for cleaning dyes from wastewater. (Liu, Zhang, Liu, Peng & Gai 2016.)

Another experiment utilized fish waste for the denitrification of wastewater in recirculating aquaculture systems. In these systems, the ammonia and nitrite, which is toxic for marine life and decreases the oxygen content of water, is converted into nitrate. However, only a limited amount is necessary, and the rest is usually discharged into the ocean, where large amounts of it degrade the water quality. The experiments were done in synthetic seawater, where sea salt, sodium nitrate, and potassium dihydrogen phosphate were added into tap water. The experiments were carried out in glass vessels that had been treated with nitrogen to remove any oxygen from them. It was found that using fish waste in water treatment decreased organic waste in the discharge of synthetic seawater, and it helped to control the nitrate levels. To remove more nitrogen, an additional step of denitrification in the aerobic environment is required, and a method to remove the fine solids in the fish waste. (He, Zhang, Main, Feng & Ergas 2018.)

A research group tried to find out if using molasses could replace sodium acetate as a carbon source for bacteria that were used for wastewater treatment in a fish farm. The fish waste was obtained from the surface of a drum filter that was removing solids from the water, leaving the fish tank. The slurry was pumped into a bacteria reactor, and pure oxygen was added into it. The molasses were added after diluting them in distilled water until a certain concentration of carbon per liter was reached. The removal of inorganic nitrogen and some of the phosphate was observed, and they delivered a performance that was comparable with the commercial alternative sodium acetate. The source of carbon proved to be less of an issue than the amount of carbon added. The greatest impact in reducing wastewater emissions is achieved if the bacteria is collected after wastewater treatment and used again. (Schneider, Sereti, Eding & Verreth 2006.)

A research group combined the solid waste discharge from a marine recirculating system to feed worms that would then grow and consume the waste. In the experiment, the worms were divided into two groups, one got only the commercial diet and the other one got the commercial worm diet and waste. The worms getting waste proved to grow bigger, and this was observed, because of the higher protein content in the waste. One option is to feed the worms with the waste of the fish, and when the worms reach their peak, they can be fed back to the fish. All in all, it was discovered that worms could be used in converting solid fish waste into biomass. (Brown, Eddy & Plaud 2011.)

One research group tried to develop a combination of sewage and fish waste into an adsorbent for the removal of antibiotics. The estimated production costs are similar to general-purpose activated carbon.

The fish waste and sewage sludge were dried at 120 degrees. Then it was homogenized into micrometer range. The materials were carbonized in a nitrogen-rich atmosphere for one hour and the temperature was increased by ten degrees each minute. The adsorption capacity improved with the rising temperatures. The best results were delivered with fish waste, which was rich in calcium due to the bones of the fish, pyrolyzed at 950 degrees, and the activity of the adsorbents was higher than that of activated carbon. The capacity was lower, but the added benefit of waste turned into something useful can compensate for that. (Nielsen & Bandosz 2016.)

#### 4.5 Nutrients & Peptides

Another research saw the discarded fish waste as a potential raw material to produce fish oil. Here, the researchers tried to extract poly-unsaturated fatty acids from the fish oil with a process called chemical alcoholysis. First, the oil which was acquired from the industry was neutralized and bleached to ensure sufficient chemical and physical properties. The chemical alcoholysis can also be called transesterification, where the oil was brought into contact with alcohol to create esters and glycerol. The final product was extracted by mixing some water with oil and hexane. After the hexane and oil were separated in a separation funnel, the oil was ready for analysis. After analysis, the oil was deemed to be good enough for later applications as a supplement in diets. (Nascimento, Bermudez, Oliveira, Kleinberg, Ribeiro, Abreu & Carioca 2015.)

The concentration of crude fish oils for nutritional purposes can be carried out in several ways. The greatest challenge is the oxidation of lipids, which degrades the nutritional value and the composition of the fish. The first method is to separate the oil into two fractions with organic solvents. The part with a higher melting point will crystallize, while the liquid part containing all the wanted substances remains. Another method is to separate the parts by mixing the reagent into urea and letting it capture the unwanted part, which will then solidify, and then it can be removed, leaving behind the liquid with the desired products. The yield is greater in the latter method because the crystals that were made in the urea complexation are more thermally stable, and that is why the filtration can be carried out in higher temperatures. A popular industrial process, distillation, starts by esterifying the fatty acids in the lipids and then the actual distillation takes place in a vacuum and in a low temperature to enable the separation of the fatty acids. Another way is to utilize supercritical extraction, where first the desired substances are extracted and then they were separated from the supercritical matrix. Usually, carbon dioxide is used as the supercritical solvent, and its consumption is the main drawback of this method. Enzymatic processes use specific enzymes to carry out the process, but here the main problem lies in

the reagent itself, which is so heterogenous that finding the right enzyme is problematic. (Morales, Munio, Galvez, Guadix & Guadix 2013.)

Hydrolysates that were extracted from fish waste, which have specific peptides contain ones that stimulate growth. These could be fed to aquaculture; however, the change observed in the experiments was not significantly different from the control group. Some can enhance enzymes being emitted into the gastric system, but no clear correlations could be established. Some of the peptides can boost the immune system of the fish, specifically the part that is non-specific, and the peptides could be added directly into the feed to boost the immune system of the fish. Other peptides can regulate appetite and have great potential in the development of weight control medication. The peptides that are manufactured are dependent on the fish waste type, species, enzymes, and reaction parameters of the hydrolysis. However, more studies are required to fully understand the behavior of these hydrolysates. (Ravallec, Cudennec & Dhulster 2013.)

There are a few options to treat fish waste and to transform it into food or a nutrient. One option is to convert fish waste into minced fish and then use it in creating food. The largest problem in this approach is the appearance of the fish mince, which is degraded by pigments that were mixed in the flesh during mechanical separation. Also, sausages can be created from fish waste and patties for fish burgers, where half of the fillet could be replaced with fish waste without any negative impact observed. Another class of food is the so-called gelled products, of which surimi is a great example of washed fish mince. Under-utilized fish species have been used in this approach. The main problem in this approach is the new washing methods since the conventional ones do not work that well, and they require additives to pass as food grade. The alternative approach is to extract the nutrients from the fish waste, like lipids and proteins. (Cardoso & Nunes 2013.)

This approach is supported by the fact that creating food products from fish waste are undermined by the contents of the fish waste itself. The proteins isolated can absorb more fat, but they cannot emulsify as much as, for example, egg white powder. Oil can also be extracted from fish waste, and here, using enzymes is preferred against choosing thermal treatments. Various enzymes, peptides, chitin, and its derivatives can also be extracted. However, the commercial feasibility of the extraction is impacted too much by the seasonal variety and location-dependent catch. Especially in the extraction of high-value products more steps are required which is a challenge for the industry because each additional step in the process costs money. (Cardoso & Nunes 2013.) In a laboratory experiment, rats were fed with fish oil that had been manufactured from visceral fish waste. Here the lipids were extracted with fermentation or enzymatic hydrolysis because of their ecofriendly nature. The greatest interest in this research was to find out if cholesterol could be lowered in the rats. The visceral waste was combined with bacteria that had been grown for one day after it was minced and cooked at 85 degrees for 15 minutes. Other substances added were dextrose and salt. The mixture was placed in a sterile, airtight container and was left to incubate for two days. The slurry created was then centrifuged, and the top layer of oil was used for the experiment. The enzymatic hydrolysis used the same cooked minced fish waste. Then water and enzymes were added, and the mixture was homogenized. After this, it was incubated for two hours at 40 degrees, with periodical shaking of the container every 10 minutes, and after centrifugation, the top oil layer was removed and used for experiments. No harmful impacts were noted in the rats, and the positive effects were similar to commercial fish oil; however, more studies are needed to find out if this option can be used in the manufacture of nutrients. (Rai, Bhaskar & Baskaran 2013.)

One option to utilize fish waste is to use the peptides within and use them because they lower blood pressure. This could be an alternative to synthetic medication, since the peptide in question inhibits one enzyme from working, which in turn lowers blood pressure. These peptides are also regarded to be much safer than conventional drugs from the pharmaceutical industry. They can be extracted from several species, such as Sardine, Tuna, and Chum salmon, from their respective hydrolysates. For example, in the case of Chum salmon, the proteins in the defatted muscle were hydrolyzed, and the respective hydrolysates were tested in lab rats, whose blood pressure dropped. It stayed on a low level for eight hours after consuming the medicine. In a case study, the by-catch of the Mediterranean region was defatted, and water was removed, before subjecting them to hydrolysis. The material was warmed in a water bath for half an hour and later pressed three times until a final pressure of 150 bar was reached. After that, the press cakes were ground and homogenized with demineralized water until a suspension was made. Then enzymes were added, and hydrolysis was carried out. The samples were analyzed after deactivating the enzymes with heat treatment. The samples could have peptides to lower blood pressure, but the specific active peptides need to be identified in future research. This is essential so that they can be fractionated and purified to enable the production of food or medicine grade concentrates. (Galvez et al. 2013.)

The peptides extracted from fish waste can also be antimicrobial in nature. These are called AntiMicrobial Peptides (AMP); however, there is no standardized assay to evaluate this. These are usually divided into four classes based on their composition or origin: linear helical AMPs, cyclic cystein-rich AMPs, AMP fragments of abundant protein, and Bactericons. Linear helical AMPs destroy microbes and fungus, and they also play a part in the immune function control. The cyclic ones act in iron metabolism, and due to their signature structure, they also have antimicrobial traits that are more specific than in their linear counterparts. AMP fragments are remnants of protein hydrolysis. These serve as an add-on to improve the existing immune defense. Bactericons are perceived as the first-response team when the immune system of the fish is attacked. All four can exist in the same fish and are usually located in the gills, skin, or the stomach of the fish. The research group suggested extracting AMPs from fish waste since they are in the part of fish that is being discarded right now. (Desriac, Jegou, Brillet, Chevalier & Fleury 2013.)

## 4.6 Biofuels

One way to treat fish waste in general is to convert it into liquid fuels and activated carbon. More specifically, the objective of this research was to make biodiesels from fish oil, turn de-oiled fish waste into bio-oil with pyrolysis and then use the bio-char that was left behind in the pyrolysis and transform it into activated carbon. The fish waste was first de-oiled in a boiling water tank to separate the fish waste from the oil and then squeezing the oil away from the fish waste. The crude fish oil was then screened and dried to create refined fish oil that could with the help of an alcohol and potassium hydroxide, be transesterified to create biodiesel. After the oil removal, the fish waste was subjected to pyrolysis treatment to further divide the fish waste into pyrolytic oil and char. The char was washed with acetone, then oven-dried, broken into smaller pieces, and sieved. The material caught on one of these sieves was converted into activated carbon with the steam activation method. The char was heated step-by-step with a constant flow of steam in order to activate it. The parameters of the biodiesel were within the limits of the American biodiesel standard. The key figures of de-oiled fish waste were similar with other bio-oils made from other types of biomass, and therefore it could be applied in the production of bio-oil. The product yield can be influenced by altering the temperature, time, or the particle size in the pyrolysis process. The greatest challenge plaguing the direct application bio-oil as a fuel in diesel engines is its low pH value. (Abdelrahman, Adnan & Hamid 2017.)

Another research topic focused on biomass and its applications as biodiesel. One of these biomasses that was researched was fish waste. In general, biomass can be converted into fuel either by distilling it to make ethanol or esterifying it to make bio-diesel. Since normally half of the weight of the fish landed is not suitable for human consumption, it will be moved into an effluent stream. This stream contains among other fish parts such as skin, liver, viscera and fecal coliform from sea birds. As highlighted before, the waste is usually, if not deposited in a landfill, converted into fish meal or fish oil. Fish meal production processes are limite due to the low value of fish waste and the production of fish oil by the energy costs associated with the cooking process. (Jayasinghe & Hawboldt 2012.)

Crude fish oil has been mixed in diesel oil, but either the properties did not differ from the original enough, or the fish oil started to crack during the combustion. Fish waste requires various steps to become biodiesel. Some processes create glycerol as a by-product, and other processes create biodiesel that is more acidic than what the norm allows. The high viscosity makes the application of bio-diesel problematic, especially in colder climates, and even though the properties are very close to normal diesel, the production creates products such as glycerol, which is hard to sell due to massive supply and low demand. The combustion lowers the  $CO_2$  emissions but increases the  $NO_x$  emissions. (Jayasinghe & Hawboldt 2012.)

One study carried out in Tunisia tried to discover the optimal pyrolysis treatment for waste fish fats and to turn it into biofuel. Due to the heterogenic nature of the waste, it has a clear impact on the quality of the fuel. This is because the waste itself has many different functional groups. Here the sample was pyrolyzed at 500 degrees, and then the condensate was captured and separated into an aqueous fraction and a bio-oil fraction. After analyzing the sample with gas chromatography and other analysis methods, it was discovered that biodiesel had still many functional groups with oxygen, which meant that future storage would need to prevent the fuel from oxidizing. Also, the oxygen in this biodiesel decreases its heating value. Besides, the viscosity of the fuel was quite high, so high that it needs to be mixed with normal diesel or treated with catalysis to reduce the viscosity. The remaining challenge is the inclusion of acids in the fuel that could corrode the engine if left untreated after producing the biodiesel. (Kraiem, Hassen-Trabelsi, Naoui, Belayouni, & Jeguirim 2015.)

Turning fish waste lipids into biodiesel in high temperatures and using clay as porous material in a process called thermally-induced transesterification was the research project of one group. The porous material is needed to provide the environment for methanol and fish waste to crash into each other. The high temperature accelerates the reaction rate and therefore ensures high yield. Clay was chosen because of its low cost when compared with the prices of commercial alternatives. The fish waste that originated from mackerel was dried at 80 Celsius degrees for one day, and the lipids were extracted with n-hexane by letting them react for one day at 75 degrees. Methanol and potassium hydroxide were mixed to catalyze the transesterification and brought into contact with the lipids in the pores. This process had the challenge that the yield increased with increasing the temperature. However, after a

certain temperature, the yield decreased due to the thermal breakdown of the esters. If conducted at the right temperature, the lipids from fish waste could be used for biodiesel production. (Jung, Oh, Park, Lee & Kwon 2019.)

One study tried to find out the potential of fish oil extracted from fish waste and turning it into biodiesel. The 10 kgs of fish waste used in this experiment were cut into smaller pieces and dried in an oven for three days. After that, their size was reduced in a mortar. The fish oil was extracted with nhexane, and it was kept warm at 60 degrees for 3 hours. The extract and the n-hexane were cooled to room temperature and filtered. Then the fractions were separated, and crude fish oil was obtained. Then the fish oil was prepared for transesterification by mixing it with methanol. A little bit of sulfuric acid was added to act as an acidic catalyst. The process was done at 60 degrees for 3 hours, and after it, the oil was washed with distilled water. In turn, this mixture was dosed with sodium anhydrous to remove water, and then the oil was filtered to remove any leftover sodium anhydrous. Now the oil was ready for transesterification after cooling down. There it got separated into two different layers, the upper one containing biodiesel, and the bottom one was glycerol, which was discarded. The biodiesel was washed with purified water, and then the mixture was warmed and separated. The oil was collected and treated as before with sodium sulfate anhydrous. Free fatty acid removal in the first step is necessary because otherwise soap will be created due to the large amount of free fatty acids in fish waste. Biodiesel was created by adding methanol and sodium hydroxide as an alkaline catalyst, and it could be produced even from low-quality fish waste. (Amira, Nor, Nur, Siti, Khairunissa & Nur 2018.)

One experiment tried to determine the effect of various parameters in the biodiesel yield, for example, temperature, the ratio of methanol to oil, and reaction time. Fish oil made from fish waste may not reach an edible quality, so an alternative application for this fish oil is to transesterify it into biodiesel. Direct application of fish oil in furnaces can create large amounts of particle emissions, and the carbon from the fish oil may deposit on fuel injectors. The high content of free fatty acids can cause the building of soap, so these free acids needed to be converted into esters, which could then be separated from the fish oil. The fish oil was stirred with sodium hydroxide and methanol, and it was then tested in various conditions. The resulting alkyl esters were washed two times with distilled water to refine the fuel, and then it was vacuum filtered through sodium sulfate to remove excess moisture. The reagent of fish oil from fish waste was turned into biodiesel with the highest yield being obtained at 40 degrees, with one-and-a-half-hour reaction time and in a ratio of 9 parts of methanol to one part of fish oil. The largest problem requiring more research is the low oxidative stability, which directly reduces the shell life of the produced biodiesel. (Garcia-Moreno, Khanum, Guadix & Guadix 2014.)

The properties of diesel originating from fish waste were researched in a reactor. Experiments were carried out, because of interest in reducing the dependency on fossil fuels and because spills of biodiesel are less hazardous in the environment. The experiments were carried out with refined biodiesel, undistilled biofuel, and standard diesel fuel. The process to make the biofuel was pyrolysis, where the fat was heated from 350 to 380 degrees with a rate of 2 to 3 degrees per minute. When the vapor left the starting position, it interacted with the catalyst surface when it went up and, in the end, it was divided into three fractions. One with the pyrolysis water second the liquid fraction that kept on vaporizing until the temperature reached 400 degrees and the third fraction containing the biofuel. The biofuel was distilled to remove the most volatile compounds and to increase the flashpoint in the process. Both biofuels generated exhaust gases of lower temperatures than normal diesel and their NOx emissions were higher than diesel. Biofuels generated less unburned hydrocarbon emissions and carbon monoxide than conventional diesel. Both biofuels created more carbon dioxide than oxygen emissions, but they did not alter the number of particles emitted. The biofuel remains an alternative. However, this was only a lab-scale experiment, and more research needs to be carried out in order to make industrial-scale applications possible. (Varuvel, Mrad, Aloui & Tazerout 2017.)

A research group conducted experiments with the goal of developing biodiesel from salmon oil, which has less lipids that the industry needs. First, the free fatty acids in the fuel were esterified to reduce the acidity of the compound. The salmon oil reacted then with methanol, and sulfuric acid was used as a catalyst, and the mixture was stirred at 300 rpm for one hour in 60 degrees. Then the mixture was separated in a separating funnel for 60 minutes, and the separated esterified fish oil was brought into contact with methanol. Sodium hydroxide was used as a catalyst. The products were settled in a funnel for 60 minutes to separate the glycerol, and then the alkyl esters were purified by washing and drying them. Had the amount of catalyst been lower in the second step, a higher FAME yield could have been reached. The viscosity could be reduced further by blending biodiesel into normal diesel. The oxidative stability needs to be improved with antioxidants before commercial applications. (Khanum, Garcia-Moreno, Guadix & Guadix 2013.)

Another research group applied fish waste as catalysis in the production of biodiesel. This fishbone waste would catalyze the reaction that turns cooking oil waste into biodiesel. The process was carried out in different temperatures, and the ratio between methanol and oil and the amount of catalyst was varied. The fish bones were washed with water, ground, and sieved to separate them according to size. The bones were dispersed into nitric acid for a few minutes, and they were then rinsed with water. After that, they were dried in an oven for one day, and then they were calcined in a furnace. The biodiesel was manufactured in a batch reactor. The product and the catalyst were separated by vacuum filtration,

and the liquid was separated into two parts; one having the biodiesel and one having the glycol. The biodiesel was washed with warm water and to remove any impurities in it. This methanolysis reaction was most successful with 86 % yield when the reaction mixture was stirred in 300 rpm. There were 18 parts of methanol for each part of the oil, and the reaction temperature was 65 degrees and the reaction time was 2 hours. (Sulaiman & Amin 2016.)

Another study researched the possibility of producing biogas and using it in energy production. The method of choice was anaerobic digestion without oxygen to generate methane. Depending on the manufacturing method, different yields of methane were expected. With different concentrations of solid material, it was found that significant amounts of methane could be accumulated with 1 % total solids concentration, and more than 200 households could use the gas produced for cooking. (Cadavid-Rodriguez, Vargas-Munoz & Placido 2019.) One option to improve the process is to introduce bamboo hydrochar to prevent the fat in the fish waste from inhibiting the process. The hydrochar was made by drying the bamboo powder overnight in an oven. Then it was poured with with deionized water into a reactor. Then with an increment of 3 degrees each minute, it was warmed into various target temperatures. Then it was cooled to room temperature, and the gaseous material was let out. After filtering in a vacuum, the hydrochar was ready. The solid material mixed in different ratios was placed in tap water, and it was left to digest for five consecutive days until no biogas production was observed. It was discovered that 1-to-2 ratio of hydrochar mixed with fish processing water at the right temperature increased yield the most (Choe, Mustafa, Lin, Xu & Sheng 2019.)

#### **5 CONCLUSION**

The fishing industry creates a lot waste that is right now either burned in the ship's engines, dumped back into the ocean, or disposed of in landfills. (Ghaly et al. 2013). However, throwing fish waste untreated back into the water creates a disturbance in the ecosystem; burning it can be harmful to the engine in the long run, and the costs of landfill disposal can be quite high. The most common approach nowadays is to convert fish waste into fish meal or fish oil; however, these methods are quite expensive, and that is why fish waste usually ends up being thrown away. The most significant challenge in treating fish waste is its heterogenic nature; one never gets the same catch twice. This makes the possible applications tricky because the catch itself heavily influences them. Turning fish waste into feed for aquaculture or livestock is feasible due to the high content of nutrients in the fish waste. Fertilizers made completely or partly from fish waste can function as organic alternatives to replace commercial ones. The fish oil that originates from fish residues can be extracted with enzymes, but due to the varied nature of residues finding the right enzyme can prove to be very challenging.

Hydrolysates can also be manufactured from fish waste. However, according to the scientific articles referenced in this work, some do not work enough, so that a correlation could be proven, whereas others could have some potential in the pharmaceutical industry, more specifically, in the production of weight control medication. Some hydrolysates could be used in the food industry, but their bitter taste plagues them, and therefore, they require more steps to negate the bitterness. The fish waste can also be minced and served for consumption, and in the research it was determined that half of the meat could be replaced by fish waste without impacting the quality too much. Enzymes could also be extracted from fish waste. However, in the case of extracting one for the production of cheese, the main problem was that the cheese manufactured was not desired by the consumers, and it could not hold the moisture inside it like cheese made commercially. For dehairing in the leather industry, enzymes were discovered, and where they could make a positive impact by delivering the same performance as other enzymes usually used, but delivering a smaller pollutant load in the process.

Biomaterials and biofillers are one application field of fish waste. When turning pure fish waste into packaging material and while they were on the same level with their commercial counterparts, they lacked in keeping the moisture inside the packaging, so they require more additives before any commercial application can take place. Fish scales could be used in composite materials to improve their biodegradability; however, they decreased the strength of the material also.

If the fish scales are used instead in starch foam, the addition makes the material more resistant against water, and it also has better thermal stability. If pure gelatin is extracted and used, it has the same challenges, with holding moisture inside the package, and it is not strong enough to be used directly. With the addition of the right substances, they could make a difference in the future. Different parts of fish waste could be used in manufacturing sorbents for wastewater treatment. For example, bones can be utilized to remove heavy metals, total fish waste to remove azo dyes, and fish waste combined with sewage to remove antibiotics. Other wastewater treatment applications use fish waste as a feed for bacteria, which in turn clean wastewater. Fish waste can also be used in feeding worms that would consume it, grow, and once they would reach a specific size, they would be fed back to the fish. Fish waste could also be used to denitrify wastewater.

The peptides within the fats can be extracted, and some of them have unusual properties, for example, they lower blood pressure (exact peptides need to be identified) and some help in destroying bacteria. Biofuels are another way to process fish waste; also, one research group made activated carbon out of it. The problems shadowing this approach are the acidity of bio-oil, the possibility that the oil starts to crack when in combustion, its high viscosity if not blended with conventional fuels, higher NOx emissions, oxygen in the fuel that lowers its heating value and the risk of fuel oxidizing, which reduces its shelf life. The oxidizing can be controlled with antioxidants. Fish waste could also be used as a catalyst to make biodiesel, or it can be turned into biogas by anaerobic digestion. All in all, fish waste has much potential in a circular approach, but it requires more research to be industrially feasible. In figure 7, various ways to convert fish waste into something more valuable are shown.

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