Pelagic fish oil

– methods for extraction and purification

Pelagisk fiskolja – metoder för extraktion och rening

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Abstract

The Pelagic fishes are a wide group of fishes that live in the open water. They could be divided in two groups depending on the size, the small forage fishes and the large predators. These fishes swim regularly and have muscles with high lipid content. There are two types of muscles in the fish. The red muscle that are used for slow and medium speed. The white muscle is used in high speed.

Fish oil are extracted using different techniques. Wet pressing uses four steps. Cooking, pressing, decantation and centrifugation. It is a traditional method. Solvent extraction is performed with organic solvents. Supercritical fluid extraction uses the properties of the supercritical fluid to extract different substances. Enzymatic extraction uses the enzymes to break down the structure of the fish.

Fishes are also a good source of healthy polyunsaturated fatty acids, such as eicosapentaenoic- and docosahexaenoic acid. Pollutants in the water easily end up in the fish. These substances accumulate in food chain and are poisonous for humans.

The crude oil contains impurities and has to be purified before consumption. There are several steps and methods to perform the purification. One common method is degumming, neutralization, bleaching, deodorization and winterization. These steps remove phospholipids, free fatty acids, metals, pigments, ions, metallic complexes, oxidation product and waxes. A combination of activated carbon and supercritical fluid extraction could remove pollutants with 100 %. Urea fractionation, chromatography and molecular distillation are methods to concentrate the refined oil.

Keywords: Pelagic fish, muscle, lipids, PUFA, fish oil, pollution, purification, extraction.

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Abbreviations

ADP	Adenosine di phosphate
ALA	α-linolenic acid
ATP	Adenosine tri phosphate
CO_2	Carbon dioxide
DHA	Docosahexaenoic acid
EFSA	European food safety authority
EPA	Eicosapentaenoic acid
EU	European union
FFA	Free fatty acids
LA	Linoleic acid
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-p-dioxin
PCDF	Polychlorinated dibenzofuran
PUFA	Polyunsaturated fatty acids
SFE	Supercritical fluid extraction
WHO	World health organisation

1. Introduction

Some nutrients are necessary to ingest for humans to live. One of them are the polyunsaturated fatty acids, such as omega-3 (Livsmedelsverket 2020a). Fish has been an important source of protein for humans for a long time. There are archaeological excavations that proves that fish were an important source of nutrients already 10 000 years ago (Boethius & Ahlström 2018). The pelagic fishes are today one of the most commonly consumed fishes and has a high amount of oil in their flesh. The word pelagic means sea or open ocean. The pelagic fishes spend their life between the surface of the water down to 200 meters deep, often in the middle and upper part. This group of fishes exist in a wide range of sizes from the large dolphinfish to the small anchovies (Douglas 2012).

The human impact on nature has caused heavy metal, PCB and dioxin pollution in water and ground. These chemical substances accumulate in the food chain and are toxic for humankind to ingest. It could affect the hormone system, nervous system, reproduction and also cause cancer (Livsmedelsverket 2019, 2020b). Fished fish can contain too high levels of these potentially toxic substances. It is possible to extract and purify the oil from the fish. One of the challenges during these procedures is to maintain the quality of the end-product. These purification processes must have minimum effect on the stability and quality parameters of the oil. With this in mind, there are several different steps and methods of purification. Each step extracts specific substances to provide a good quality at the end product.

1.1. Aim

The aim of this thesis is to collect and summarise information about pelagic fishes and how to produce and purify the oil from the fish.

2. Method

The material in form of scientific books, journals and articles were found on AFSA, FSTA and SLU's library search site Primo. Key words used in the research were different combinations of; "pelagic", "fish", "lipid", "muscle", "contamination", "pollution", "purification", "extraction", "methods", "fish oil", "fishmeal", "PCB", "heavy metals" and "dioxin".

3. Pelagic fishes

3.1. Background

The pelagic fishes do not consist of a specific breed or gene. They are rather a large group of fishes that lives in the open water of both lakes and oceans (Vlieg *et al.* 1993; Sandström *et al.* 2014; Van Beveren *et al.* 2014). They are often acting in schooling groups swimming together in one direction (Alder & Pauly 2006). The pelagic fishes could be divided in two categories after size. Small and large fishes see Table 1.

Table 1. Examples of common small and large pelagic fishes.

Small pelagic fish	Large pelagic fish
Mackerel	Tuna
Herring	Swordfish
Sardine	Salmonids
Anchovies	Pelagic sharks

3.1.1. Small pelagic fishes

The small pelagic fishes could be called the forage fishes (Pikitch *et al.* 2014). They are an important component in the sea, both for the ecology, sustainability and the human economy. They maintain an role to keep the ecosystem under water intact and are essential constituents in the trophic system (Shephard *et al.* 2014). They feed on the lower tropic level organisms and are eaten by the upper level tropic predators (Pauly *et al.* 1998; Cury *et al.* 2000). The small pelagic fishes are an important catch for fisheries and accounts for 30 % of the catch weight globally. It fulfils the need of food for many people in the world and it is important to avoid overexploitation (Essington *et al.* 2015).

3.1.2. Large pelagic fishes

The large pelagic fishes are predators with a high tropic status. They have a large body size and a high metabolic rate in comparison to the small pelagic fishes. The predators search in long distances for their target. The body has evolved during evolution and these fishes have big amount of red muscle to be able to swim long distances (Croll & Tershy 2008).

3.2. The fish muscle

Fishes spend their entire life in the water. Most of the time swimming actively. The activity from the muscle requires energy and the body moves in one particular way to travel forward. Almost like a fluttering flag (Müller 2003). The mechanical way to move the body starts with an sideway rhythmic movement that then rotates backwards (D'Août *et al.* 2001). Many fishes take help from vortices to move, to minimize muscle activity (Liao *et al.* 2003).

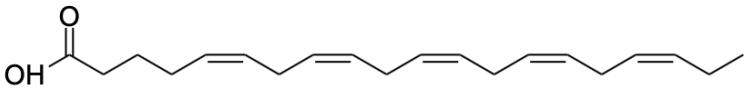
The swimming muscle is divided in two different structures. The red muscle that helps the fish move in slow- and medium speed and the white muscle that achieves a fast speed (M. D. Rayner & M. J. Keenan 1967; Johnston *et al.* 1977). The main causes of these differences in muscular structure is mitochondria content, blood supply and enzymatic activity, especially ATPase. The red muscle needs a constant source of energy and has a good blood supply, enzyme activity and mitochondrial content. The myofibrillar ATPase activity is low. Both carbohydrates and lipids are used as an aerobic metabolic energy source. The white muscle uses anaerobic glycogenolysis for energy. They have a low content of mitochondria and blood supply. Although the ATPase activity is high to be able to quickly catalyze the reaction of ATP becoming ADP (Johnston *et al.* 1972, 1977; Mosse 1979). The white muscle is mainly used when the fish is threatened and needs to flee. The use of glycogen is draining energy and it takes days to recover (Douglas 2012).

3.3. Lipids from fish

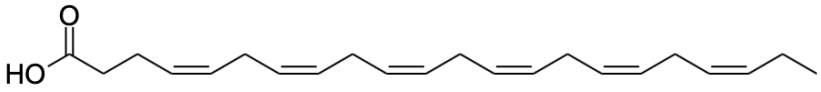
Fish is a good source of numerous nutrients but mainly polyunsaturated fatty acids (PUFA) such as α-linolenic acid (ALA), linoleic acid (LA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Maestre *et al.* 2011). PUFAs are essential to ingest for humans to live. A regular intake of these lipids could decrease the risk of coronary artery disease, diabetes, arthritis and inflammatory disorders (Simopoulos 2000). Especially EPA and DHA that is long chain PUFAs. Humans are not able to synthetize amino acids higher than 18 carbon. It is important to ingest

these fatty acids regularly the whole life. They are involved in different processes such as development of the cell membrane, retina and foetuses during pregnancy (Swanson *et al.* 2012).

The PUFAs have two or more double bonds in the carbon chain. The double bonds have cis-conformation in natural fatty acids, see Figure 1. These double bonds say much about the properties of the fatty acid. The length between the terminal carbon to the closest double bond decides the omega number. The amount of double bonds determines the melting point of the fatty acid. The more cis-configurated double bonds, the lower melting point (Coultate 2016).



Eicosapentaenoic acid (20:5, n-3; EPA)



Docosahexaenoic acid (22:6, n-3; DHA)

Figure 1. This is the structural formula of two common PUFA's, EPA and DHA. The double bonds are present in cis-conformation.

The lipids could autooxidise and lower the quality of the oil. Reactive free radicals initiate the oxidation of the oil. These reactions could be light induced. This could end up in rancidity with an off flavour to the oil and reduced shelf life (Coultate 2016)

The European food safety agency (EFSA) recommend a daily intake of PUFA, see Table 2 (European Food Safety Authority 2009).

Table 2. Daily recommended intake of PUFAs, according to EFSA.

Fatty acid	Recommended daily intake
ALA	2 g
EPA & DHA	250 mg
LA	10 g

The lipid content in the pelagic fishes mackerel and sardines do vary along with the nutrition the fish itself will ingest, but also other external & internal influences. After a season with loads of feed, the levels of oil in the muscle increases in

comparison to season with less feed. There is also a difference of oil content in the red and withe muscle of the Mackerel. The red muscle contains higher amounts of phospholipids and the white muscle higher amounts of triglycerides (Ackman & Eaton 1971; Ben Rebah et al. 2010).

3.4. Contaminants

The tropic system under water could lead to problems regarding accumulating pollutants in the food chain. Fishes could contain great amounts of dioxins, PCB and heavy metals such as mercury. Many of these pollutants are lipophilic and end up in fat-rich parts of the fish. These substances are harmful for humans in high amounts.

3.4.1. Dioxins and PCB

Dioxins and polychlorinated biphenyl (PCB) are organic environmental pollutants that are difficult to extinguish. Dioxins are produced industrially as a by-product of other chemically manufactured substances. Dioxins consists of hundreds of different chemical combinations of the dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF). PCB is an industrially used product known for its insulating capacity. Both dioxins and PCB could affect the hormonal system, development of the central nervous system, cause cancer and affect the fertility of humans (Livsmedelsverket 2019).

It is important to avoid intake of contaminated food since dioxins and PCB is fat soluble and is stored in the body fat of humans and animals. These pollutants can be transferred to the offspring during pregnancy and from breast milk. The intake of dioxins and PCB are highly regulated in European Union (EU) (Livsmedelsverket 2019).

3.4.2. Mercury

Mercury is naturally occurring in soil and water. Methylated mercury is naturally created out of the nonorganic mercury. This version of the substance is found in high concentrations at some waters. Fishes that lives in these places takes up these molecules and it accumulates through the tropic system. The longstanding predators are the fishes that have the highest estimated amounts in their bodies. A high consumption of mercury could damage the function of the central nervous system in humans, especially at foetuses. Mercury could be transferred to the offspring through breast milk and during pregnancy. World health organization (WHO) regulates the intake and recommends to not ingest more than 1,6 micrograms per kilo bodyweight each week (Livsmedelsverket 2020b).

3.5. Products of the pelagic fishes

The particularly high amounts of fat of the pelagic fishes encourages a high demand for this food from humans. They do contain important minerals, trace elements and vitamin A, D and E. It is an easily accessible, cheap source of energy and nutrition for the consumers. The pelagic fishes could be consumed cooked or raw. It is common to use different preservation methods, such as canning, curing or freezing (Douglas 2012).

Fish meal and fish oil are products from the pelagic fishes. Large quantities of the catch worldwide end up in this industry. It is mainly the forage pelagic fishes that is used for this purpose due to their low tropic status. Their low tropic status entails a lower amount of pollutants (Stephenson & Smedbol 2009).

4. Extraction

Fish oil and fish solids could be extracted using different techniques. The fish contain three different fractions; water, oil and solids. The extraction process is separating these three fractions.

4.1. Wet pressing

Wet pressing is a traditional method of oil extraction. It is frequently used in industries and includes four steps. Cooking, pressing, decantation and centrifugation. The cooking is performed in a cylinder chamber at temperatures between 75-100 °C. This allows the proteins to denaturate and the fat cells to break and to release the oil. Subsequently the fish is pressed until the liquid fractions are removed. The liquids from cooking and pressing are centrifuged in a decanter to separate eventually remaining solids from the liquid. And the remaining liquid is centrifuged to separate oil from the water. The oil is ready for further purification and the fish solids are mixed with the water from the previous step (FAO Fishery Industries Division 1986; Bimbo 2011).

This method has been used for a long time. The yield is fairly high in comparison to extraction with solvents. The risk of contaminated oil during extraction is particularly low, especially in comparison to other methods were the carcinogenic $CHCl_3$ could be used. The free fatty acid (FFA) content is low as well, which entails a better taste and texture. The oils do easily oxidise through the applied stress of heating and pressing. The content of DHA and EPA is contrariwise higher than the solvent extracting processes (Chakraborty & Joseph 2015a).

4.2. Solvent extraction

The meaning of solvent extraction is to extract a desired material from a solvent or solid by adding a diluent. It could be performed with numerous of different organic diluents. It relies on the chemical properties of polarity. Oil is soluble in organic diluents. This solubility regulates the extraction of oil and could separate the oil from other liquid or solid materials This method is used in industry and food

analyses. The extracted oil is not suitable in food intended for human consumption before it has undergone further purification (A. A. Bawa & O. D. Adeniyi 2006).

The chosen diluent, time and amount of sample determine quantities of diluent needed for the extraction. The fish is minced and mixed with the selected organic diluents. The mixture could subsequently be filtered or centrifuged to separate the fractions. The oil could be removed from the solvent and further be cleaned by the purifying methods. These steps could be repeated to maximize the extraction of the sample of fish (A. A. Bawa & O. D. Adeniyi 2006; Bimbo 2011).

4.3. Supercritical fluid extraction

A supercritical fluid could be any substance that behaves and have properties as both gas and liquid at the same time. This entails a good property of transport and solvent effect. The molecular density of the supercritical fluid is regulated by temperature and pressure. The supercritical state appears above the critical point of the substance. The supercritical subject can pass through both solids and liquids due to its density. Carbon dioxide (CO_2) is considered to be a good supercritical fluid for oil extraction intended for food. It is inexpensive, performed in low temperatures and non-toxic for humans. The critical temperature of CO_2 is 31 °C and critical pressure is 73.8 bar. CO_2 becomes gas and evaporates in room temperature and pressure. This method is environmental-friendly in comparison to the use of organic solvents as extraction agents (Brunner 2005; Létisse *et al.* 2006).

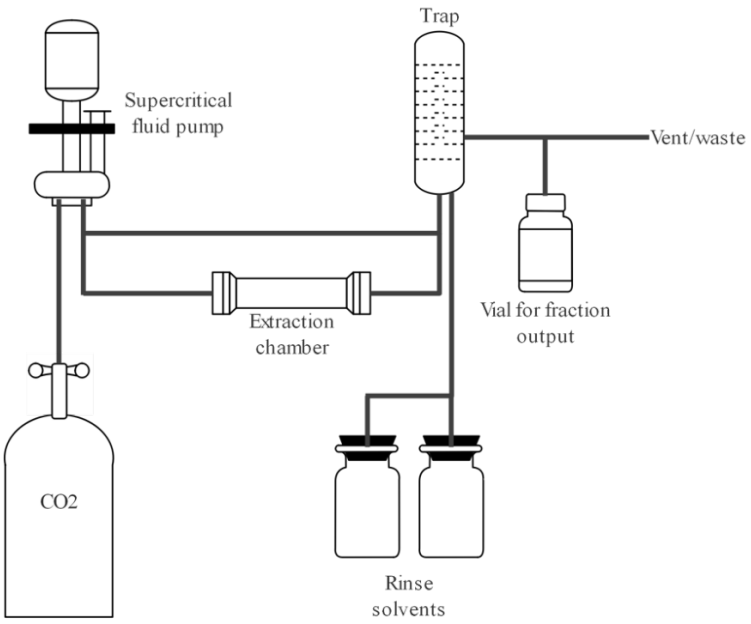


Figure 2. The flow of supercritical fluid extraction.

The method of supercritical fluid extraction (SFE) with CO_2, see Figure 2, starts with preparation of the fish sample. The fish is minced and lyophilized to minimize the water content. The sample is placed in a thimble with a heating jacket. The thimble is positioned in the extraction chamber. The extraction fluid is regulated by the supercritical fluid pump and there is a constant flow through the system. There are several valves to regulate the pressure as well. The first step in the extraction is when the supercritical fluid pass through the extraction chamber. Materials of the sample is dissolved and sticks to the trap while the CO_2 exit through the waste vent. The second step of the extraction could start. The fraction output is collected in a vial and CO_2 evaporates from the sample when it enters room temperature and normal pressure (Létisse *et al.* 2006).

4.4. Enzymatic extraction methods

Enzymes can break down the structure of the fish and enable extraction of oil. It is performed in two alternative ways. The first method is autolysis where the naturally present enzymes achieve the job. The second method is hydrolysis where the naturally occurring enzymes and bacteria are damaged through pasteurization. The desired enzymes are then added to the fish. pH is adjusted in both methods to enable the desired mechanisms of enzymes and bacteria (Bimbo 2011).

The fish silage production is a longstanding method for oil extraction. This is a type of enzymatic autolysis. The raw fish is minced, pH is adjusted, and the enzymes can start to break down the fish during a required time. The silage is centrifuged to separate the oily layer from the silage. The silage itself could be dried to form a powdered fishmeal (Bimbo 2011; Hossain & Alam 2016). This method is cheap, does not require high temperatures and requires low energy. It is not used in a big scale in the separation branch since it requires loads of time and the smell is not pleasant (Jeimmy Rocío Bonilla & José Luis Hoyos Concha 2018).

5. Purification

The extraction processes result in crude oil. This oil contains impurities such as pigments, FFA, alcohols, PCBs, heavy metals and other compounds. These impurities could be toxic for human consumption and reduce the quality of the oil. Oxidation products such as ketones and aldehydes could create off-flavors. FFA, pigments and metal salts could lower the oxidative stability of the oil (Čmolík & Pokorný 2000). There are several steps in the purification process that will extract these unwanted substances.

5.1. The five steps of purification

One common method to improve this quality parameters is to perform degumming, neutralization, bleaching, deodorization and winterization. See Figure 3.

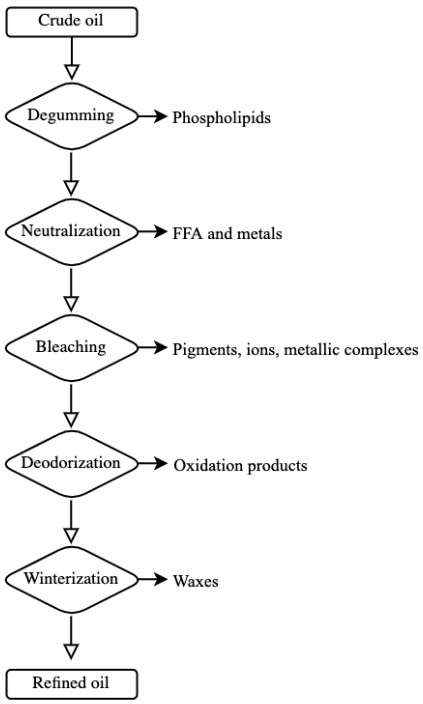


Figure 3. Flow chart from crude oil to refined oil when performing a purification. Degumming, neutralization, bleaching, deodorization & winterization.

The purpose of the degumming is to separate the phospholipids and other viscous or jelly-like substances from the rest of the oil. The phospholipids could decrease the surface tension and speed up the oxidation process. It is undesired in the end product. The degumming process is facilitated by acid that hydrate the phospholipids (Chakraborty & Joseph 2015b). The crude oil is stirred for 20 minutes at 70 °C together with the acid, for example phosphoric acid. The mixture is centrifuged when it has reached room temperature again. This centrifugation separates the hydrated phospholipids from the oil (Chakraborty & Joseph 2015b; Vida Šimat et al. 2019).

The purpose of neutralization of the oil is to remove the FFA and metals and at the same time neutralize the pH. FFA could be sorted away during the extraction processes. Adding natrium hydroxide (NaOH) induces saponification. The neutralization is performed by slowly drop NaOH solution to the oil. The oil could preferably be heated to 65 °C and stirred to speed up the reaction. When pH reaches the neutral level of 7, heating is performed to 70 °C for 20 minutes. When reaching room temperature again the oil was centrifuged to separate the neutralized oil from the layer of soap (Chakraborty & Joseph 2015b).

The purpose of the washing, drying and bleaching is to remove pigments, water, ions and metallic complexes (Jeimmy Rocío Bonilla & José Luis Hoyos Concha 2018). The step of bleaching is the most crucial step. The added bleaching agent implement the reaction of adsorption. This improves quality of the oil regarding taste (García-Moreno *et al.* 2013). The first step is to clean the oil with deionized water. The oil and water are stirred and heated together in vacuum until reaching 50 °C. Centrifugation is performed to separate the different sections of oil and water, to dispose the unwanted substances. The drying is performed at 90 °C to remove the remaining water moisture. The bleaching is achieved by adding a bleaching agent such as activated carbon powder. The mixture is then stirred together and heated in an atmosphere of nitrogen, N_2. The sample is then centrifuged to separate the layer of oil from the coal (Chakraborty & Joseph 2015b; Vida Šimat *et al.* 2019).

The deodorization process does remove the oxidation products of the oil, such as ketones and aldehydes. These products cause an unwanted taste of the product and could potentially be harmful to ingest. It is performed with a laboratory deodorizer. This process could encourage unwanted reactions itself due to the high temperatures. For example, degradation reactions or change of cis bonds to trans bonds in EPA. It is possible to control the results after this procedure. It could be done by capillary gas-liquid chromatography analysis (Ackman 1990; Berdeaux *et al.* 2007). The procedure of deodorization is performed at high temperatures (250

°C) and pressure (1.5 mbar) for 3 hours in vacuum. This procedure has to be performed without the presence of air to reduce the risk of oxygen related reactions with the oil. A steam distillation is performed and the volatile and oxidative products can leave the oil (Dudrow 1983).

Winterization is a method to concentrate a desired oil through separating the different oil components. This separation is based on partial crystallization. The different melting points of the oil regulates the crystallization. The temperature of the oil mixture is lowered so that the saturated and monounsaturated fatty acids crystallize. Their high melting temperature provide a crystallization before the PUFA's. The crystallized fatty acids could be separated from the liquid, by filtration (Vázquez & Akoh 2012).

5.2. Further methods

Besides the process of degumming, neutralization, bleaching, deodorization and winterization there are other methods available for purification and concentration of oils.

5.2.1. Activated carbon combined with SFE

This is a more specified method to remove the environmental pollutants from the fish. A combination of the two common widely used methods of activated carbon in and SFE could reduce the toxicity from pollutions with up to 100 % (Kawashima *et al.* 2006).

The supercritical fluid extraction is not only suitable for extraction of the fish oils. It is likewise useful to extract PCB and heavy metals from the oil. The different parameters in the procedure can be adjusted to maintain a separation of desired materials. The temperature range between 60-150 °C for PCB extraction and is as low as 50 °C for mercury extraction. The pressure could vary from 145-355 bar for PCB extraction and occurs at 203 bar for the mercury. CO_2 is used as extraction fluid for these extractions as well (Valcárcel & Tena 1997).

The activated carbon has a lot of small pores at its exterior. These pores provide the possibility encourage adsorption removal of other substances (Kawashima *et al.* 2006). The procedure of activated carbon treatment starts by heating the oil to 70 °C and add it with a pressure at 50 mbar to a rotary evaporator. The evaporator contains activated carbon- powder. Mixing occurs for 30 min at 70 °C. The mixture is then filtrated to remove the activated carbon and the substances attached to it (Maes *et al.* 2005; Ortiz *et al.* 2011).

5.2.2. Concentration

The purified oils are often further treated to concentrate omega oils or other specific lipids. A widely used method for omega 3 concentration is the urea adduction. Urea molecules have the ability to form complexes and crystallize with saturated and monounsaturated fatty acids. These crystallized complexes could be filtrated away from the oil. It is a pleasant method to use. It does not require any hazardous chemicals and the procedure is performed in gentle temperatures (Namal Senanayake 2010). The concentration could also be performed by different chromatography techniques or distillation.

6. Discussion

The aim of this thesis was to summarise information about pelagic fishes and how to produce and purify the oil from the fish. The pelagic fishes are valuable for humans in many aspects. Especially regarding the need of nutrients, since they contain a high amount of PUFAs. This value provides an opportunity to make money for industries. And people who don't live near water are offered the opportunity to have essential omega oils.

Different methods of extracting the oil are used worldwide. I have chosen to look into four common types of extraction. The wet pressing is traditional and a physical method where no chemicals are used. This gives a product without potentially harmful rests of other solvents. This method is frequently used in industries for fish oil production due to its simplicity. It is a relatively cheap method that gives stable results. The solvent extraction on other hand is a chemical method that leaves rests from solvents in the oil. This method is consequently not suitable for food production. It is mainly used for analytical purposes. The two remaining methods are the SFE and enzymatic method. The SFE is a well applied and flexible method that uses the physical properties of the chemical solvent for extraction. This technique requires an investment and there are relatively high running costs due to the constant need of CO_2. The CO_2 is fairly cheap itself, but the constant need of this substance gives high costs. This method has a lot of advantages and possibilities. However, the method is new and more expensive than other methods. The enzymatic method relies on the enzymes. This method doesn't require materials other than for storage, pasteurization and mincing. This method could be performed by small producers as well as individuals. But it could be hard to scale up. The disadvantage of this method is the smell. It is time-consuming, requires loads of place and not very trustable due to the different enzymes used. Regarding the environmental aspects, a review article in green methods of extraction was made in 2017. This article was based on energy consumption, renewable natural substances and providing a high-quality product without damage of the circular bioeconomic principles. All methods of extractions in my thesis were analysed in the review article. The results showed that SFE were the best green choice for extraction. The traditional methods of pressing and solvent extraction has a high

energy demand and uses organic solvents, which gives them a low environmental and ecological status (Ivanovs & Blumberga 2017).

The 5-step method of purification is a polite method to remove several different kinds of impurities. This method is widely used in the fish-oil industry and well-studied. These steps are easily performed but the question of effectivity remains. Is it viable to perform all these steps? Is it sustainable? Could it be performed in an easier way, to reduce all the different steps? SFE on other hand is a green method that has a high effectivity. The combination with activated carbon makes it even more effective and removes up to 100 % of impurities of the oil. This high-quality factor does have disadvantages such as the economic aspects. It is difficult to find statistic and information about the usage of these new combined methods. It would be interesting to look closer into the SFE and see if it could extract all different impurities. Maybe try to adjust the parameters of pressure and temperature to find the different extraction values for all possible impurities. The potential of this method seems to be infinite. It can be used both in extraction from the fish itself and to purify oils. It arouses an interest and would be intriguing to know more about.

The conclusion of this thesis is that there are several well studied and used methods of fish-oil extraction and purification. They are widely used industrially and there is a constant development to find better solutions for the environment. The method that stands out is the SFE method that really has potential to revolutionize the future with its effectivity and green footprint. It would be interesting to dive deeper into the possibilities with SFE with its advantages and disadvantages. Future research questions could be about the effectivity of the 5-step method. What method is frequently used in industries? It would also be interesting to look into the potentials of SFE.

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