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REPORT REVIEW/APPROVAL

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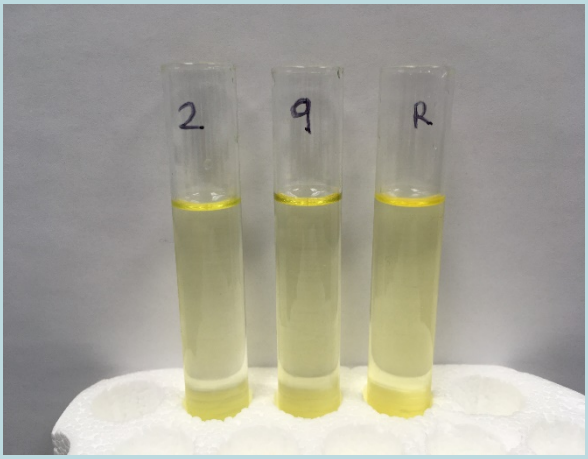

Evaluating the Natural Extraction of Oil from Cod Liver

Submitted to:
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EXECUTIVE SUMMARY

This report summarizes a process development study for the extraction of extra-virgin cod liver oil (EVCLO) on a laboratory scale. The general approach was to develop an accelerated traditional rendering process that achieves high oil yields at ambient (17°C) temperature.

Fresh Atlantic cod (*gadus morhua*) liver was collected and delivered by Jerseyman Island Fisheries Inc. to the Marine Institute following detailed handling and tightly coordinated shipping protocols aiming at providing ultra-fresh raw material. After primary particle size reduction, the fatty tissue membranes were weakened using treatments such as sonication and freezing, before the tissue was placed into a vacuum rendering chamber for 24 h. Different rendering set-ups were compared and a conceptual design of a pilot-scale chamber is presented.

The collected oil was rated based on yield, smell, color and clarity and the best oil samples were selected for in-depth analysis. The oil was of excellent quality for appearance and freshness parameters as well as for the nutritional profile (fatty acid composition and vitamin content) and heavy metal content. Elevated PCB concentrations were detected which must be removed from the product.

The report concludes with a recommendation of a phase II study to investigate process optimizations, scale-up and refining.

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ABBREVIATIONS

FFA	Free Fatty Acid
PV	Peroxide value
AV	Acid value
p-AV	p-Anisidine value
MG	Monoglyceride
DG	Diglyceride
TG	Triglyceride
PL	Phospholipids
PCDD	polychlorinated dibenzo-p-dioxins ("Dioxins)
PCDF	dibenzofurans ("Furans")
PCB	polychlorinated biphenyls
HCB	hexachlorobenzene
DDT	dichloro-diphenyl-trichloroethane
PBDE	polybrominated diphenylethers
POP	Persistent Organic Pollutants. Include: PCDD, PCDF, PCB, HCB, DDT, PBDE
HDPE	High Density Polyethylene
PVC	Polyvinylchloride
MW	Molecular Weight
MEUF	Micelle-enhanced Ultrafiltration

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1 SCOPE AND PURPOSE

1.1 Scope of Project

The scope of this study was to perform a preliminary study on the chemical-free extraction of cod liver oil at ambient temperatures and to identify refining needs through a literature review and sample analysis. This project is intended to be the first phase of a multi-year initiative that aims to lay the groundwork for the full utilization of cod.

1.2 Purpose of Project

The purpose of this project is to help the client to expand their product portfolio for ranched cod, potentially significantly increasing the resource value and improving the viability of the client's operation.

2 OBJECTIVES

The objectives of this project were:

- To perform a literature review to identify process parameters for Cold Extraction of Cod Liver Oil, membrane refining technology and quality specifications for the oil.
- To identify equipment, filter media, types of materials required for cold extraction of cod liver oil.
- To study the cold extraction method in terms of:
    a. Levels of particle size reduction
    b. Freeze-thaw cycles for oil release
    c. Removal of solids and water from oil
    d. Removal of organic pollutants using silicon based or solid based adsorbents
    e. Stabilization of oil
    f. Yield and quality analysis of the oil
    g. Residual oil in protein/water phase

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3 METHODS

After thorough literature review on cod liver handling for edible oil extraction at low temperatures, fat cell lysis methods and oil stabilization, the project team designed an extraction process for extra virgin cod liver oil (EVCLO).

In short, the process is a modified version of natural cod liver rendering. By pre-treating the livers with freeze/thaw cycles and sonication, the membranes of fat cells are weakened which results in an accelerated oil release during rendering. A vacuum is applied to the rendering chamber to inhibit oil oxidation.

3.1 Sample Collection

Fresh cod livers were collected and shipped to the Marine Institute by the client, Jerseyman Island Fisheries (JIF) Inc. Upon harvest, the fish were bled immediately and cooled in ice water. The livers were collected and cleaned from foreign matter and other tissue, including the removal of the gall bladder. Livers that were contaminated with bile were discarded. The livers were placed in clear plastic bags and sealed with minimal amounts of air trapped inside the bag. The bags were placed in a cooler on ice and shipped to the Marine Institute within 1 day of harvesting.

3.2 Primary processing

Fresh livers were received in three shipments on Dec 2019 (sample #1), Nov 24, 2020 (sample #2), Nov 29, 2020 (sample #3), and Jan 7, 2021 (sample #4). Project technicians further processed the livers on the same day. In an initial quality assessment, the sample was checked to ensure that the product temperature was below 3°C and that all gall bladders and other foreign tissue was completely removed. The livers were separated into ~1 kg portions, which were either left intact, chopped into 1 cm pieces using a plastic knife (to avoid metal oxidation), or ground in a meat grinder (5 mm plate), see Figure 1. The portions were then vacuum packed and frozen.

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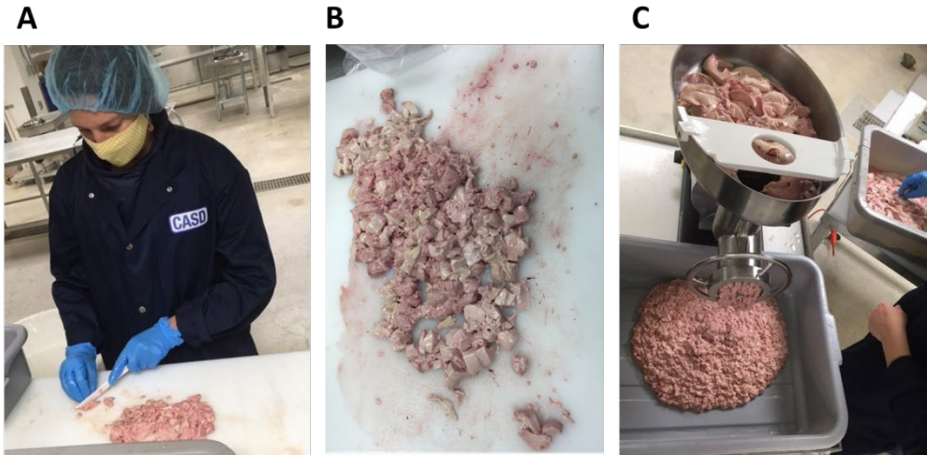

Figure 1: A - cutting livers with plastic knife; B- chopped livers; C - grinding livers through meat grinder

To freeze the product, the packaged livers were placed in a household chest freezer with an average temperature of -22°C to effect slow freezing. After the product was frozen, it was moved to a cold storage with an average temperature of -26°C. The product was held in cold storage for a minimum of 7 days before rendering experiments were conducted.

A sample of each shipment was used to determine the water and oil content method (using standard procedures AOAC 948.15 and AOAC 930.15), which was used to calculate the oil extraction yield.

3.3 Sonication

Sonication was used in some experiments to effect cell rupture and oil release from the liver tissue prior to placing the livers into the rendering chamber. The frozen, vacuum-packed product was placed in a Branson sonicator (Model CPX 2800H, output: 40 kHz +/-6%) water bath for 30 minutes (Figure 3). The initial temperature of the water bath was 18°C. After the sonication process, the water bath temperature was 25°C and the product was fully thawed.

3.4 Oil extraction

The oil was extracted from cod livers using a natural rendering approach at cool temperatures under vacuum. Prior to being placed into the rendering chamber, different treatments were employed to weaken or rupture fat cells, such as freeze/thaw, sonication, and/or homogenization. Following that, the oil was rendered out naturally over a fixed time.

The setup of choice (Figure 2) was placed in a vacuum chamber and a vacuum of -22 mm Hg was drawn on the chamber. The whole setup remained at 17°C for 24 hours.

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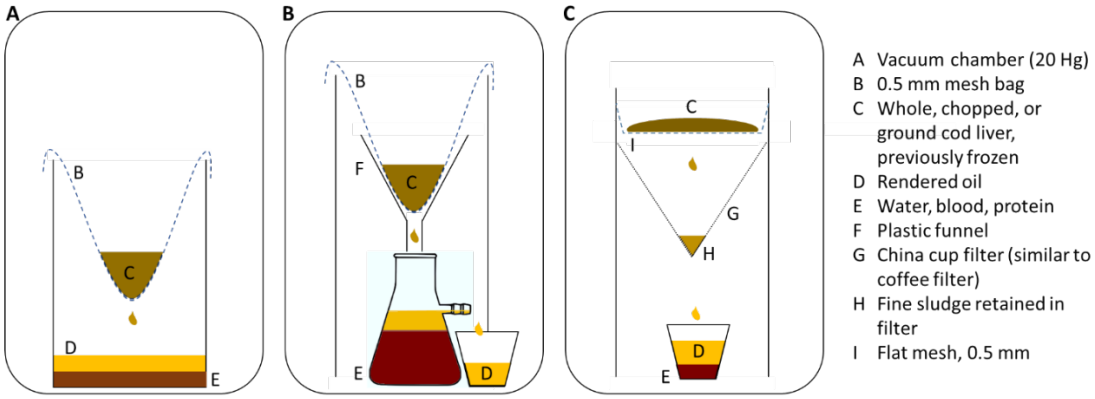

Figure 2: Schematic drawings of various oil rendering set-ups

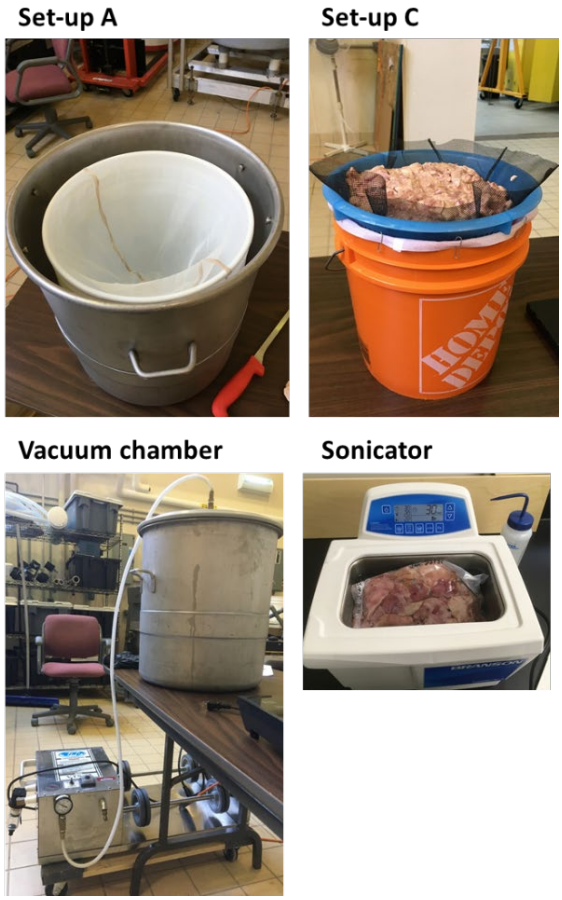

Figure 3: Sonication and rendering equipment: Set-up A – mesh bag over plastic bucket shown in the vacuum chamber; Set-up C shown without the vacuum chamber

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After 20 hours of rendering in Set-up C, the livers were gently stirred using a plastic spoon to help oil drainage before placing the setup back in the vacuum chamber. After 24 h, the experiment was finished.

The total volume of released liquid was measured (oil + water), as well as the oil volume recovered. Observations on draining, phase separation, sludge formation etc. were noted, as well as oil color, smell, appearance of the tissue before, during and after rendering.

The percent oil recovery was calculated as:

$$\%\ Oil\ yield = \frac{Oil\ weight_{\ recovered}}{Oil\ weight_{in\ 1\ kg\ livers}} * 100$$

The oil weight in 1 kg of livers was calculated from the lipid analysis of the raw livers. This yield calculation replaced the comparison of cold extraction of heat extraction as outlined in the proposal.

The collected oil was placed in a mason jar and cooled overnight at 2-8°C. The next day, the oil was centrifuged at 10,000 rpm (11.499 x g) for 40 min to clarify and to remove precipitated wax and other impurities (winterization). The clear oil phase was decanted into a fresh container, layered with Nitrogen and frozen at -80°C until analysis.

4 RESULTS and DISCUSSION

4.1 Literature review: Extraction of edible oils

Oils and fats have been extracted from plant and animal sources for centuries and have countless applications in nutrition, cosmetics, cooking and other fields. Some oils, like omega-3 rich fish oils, are particularly vulnerable to nutritional and oxidative damage during extraction and refining. Extraction and refining methods must be chosen based on the specific characteristics of the oil and the tissue that they're extracted from.

4.1.1 Vegetable oils

The biggest edible oil industry is the oil seed industry, or "vegetable oil". The most common examples are sunflower oil, canola oil, and sesame oil. Each seed has its own specifically designed process, but we will only give a very brief overview over the general process. Oil seeds contain well protected oil bodies inside the seed, which need to be released with a combination of mechanical, thermal, and solvent treatments. *(Hamm, Hamilton and Calliauw, 2013)*

The first step in the extraction process is the receiving and sampling of the material. Depending on its estimated value, suitable storage conditions are chosen. Oil seed can generally be stored for a while before processing if optimal conditions are provided. Before oil extraction, the seed must be cleaned and dehulled (if applicable). Following that, the seed is cooked at various temperatures and steam conditions to soften the hull and cell wall prior to flaking. *(Hamm, Hamilton and Calliauw, 2013)*

Flaking is the last step prior to the actual oil extraction step. The flaking process creates porous particles that allow a solvent (usually hexane) to pass through, thereby optimizing the accessibility of the oil bodies to the solvent. The seed is ground to a powder and then pressed into flakes with uniform thickness. The extraction solvent is then percolated through these flakes, dissolving and carrying away the oil contained within. Finally, the solvent is evaporated, leaving behind pure oil. *(Hamm, Hamilton and Calliauw, 2013)*

Solvent extraction is the most popular method giving the highest yields. However, it is relatively costly and requires a high capital investment. It is only cost-effective if a high daily throughput can be guaranteed.

It is important to note that solvent extraction requires a dry sample and is therefore not directly applicable to fish oils as a costly drying step would be required first.

4.1.2 "Soft" seed oil

The most prominent example of a soft oil seed is the olive and extraction of its oil has been extensively researched and optimized.

Olive oil extraction follows a completely different process from oil seeds due to its softer nature. The oil is extracted from the pulp of the fruit. Delicate harvesting is important for the final quality of the oil. Olives cannot be stored for long periods of time. After cleaning of the fruit and the removal of the stone, the flesh is crushed to a paste. Slow stirring of the paste allows oil to form bigger droplets. *(Hamm, Hamilton and Calliauw, 2013)*

Extra virgin olive oil (EVOO) is then pressed out of the paste (1-1.5 h pressing), leaving behind pomace. This cold extraction has approximately 86-90% yield. This oil is considered most nutritious and is sold at premium prices but it is also the most expensive oil due to the labor-intensive batch process. A more modern process uses a hammer mill to crush (but this forms emulsions), followed by heating (to break the emulsion) and decanter centrifugation. This process produces similar yield to batch pressing. *(Hamm, Hamilton and Calliauw, 2013)*

The remaining oil can be extracted by drying the pomace, grinding & flaking, followed by solvent percolation extraction. The final residual oil content of the pomace is <1%. *(Hamm, Hamilton and Calliauw, 2013)*

Cold pressing is not a clearly defined term and is used for a variety of conditions. For example, it does not consider the heat generated inside the press during pressing, and often just means that no external heat is applied. Cold pressed oil is usually bottled and sold with minimal downstream processing. *(Hamm, Hamilton and Calliauw, 2013)*

4.1.3 Animal fats and oils

Fat from warm-blooded animals is usually solid at room temperature due to a higher concentration in saturated and monounsaturated fatty acids in comparison to the liquid plant, vegetable, and oil seed oils. Also, the average fatty acid chain length is longer, which also increases the melting point of a fat (Adewale, Dumont and Ngadi, 2015).

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Animals store their fat in adipose tissue – fat cells specifically designed for the storage of fat. In addition, some fat is stored within muscle tissue. After mechanical particle size reduction, animal fats are rendered out using heat treatment, which liquefies the oil, and denatures and ruptures the cell walls. The oil is released into the surrounding tissue, from where it can drain. Enzyme treatment, sometimes microwave-assisted, has also been found to yield excellent results – however, industrial applications are limited to date (Adewale, Dumont and Ngadi, 2015).

Low-temperature extraction methods have not been explored in depth because of the higher melting point and the better oxidative stability of most animal fats.

4.1.4 Fish oils

Fish, as cold-blooded animals, have higher amounts of unsaturated fatty acids in their body fat, making it liquid at room temperature. While the fat is evenly distributed in some fish like salmon (*salmon salar*), cod *(gadus morhua)* store their fat almost exclusively in the liver (Aas, Kjerstad and Barnung, 2016) .

Most available fish oils are extracted using a standard wet rendering method, consisting of grinding, cooking and centrifugation (Opinion, 2010). However, extraction parameters and equipment are adjusted to the softer texture of the fish tissue, which releases oil at somewhat gentler treatment when compared to land animal meats. Enzymatic methods have been successfully developed for the extraction of fish oil, but to date, commercial applications remain limited as enzymes tend to drive up production cost.

Fish oils do have in common a faster oxidation rate due to the high amount of polyunsaturated fatty acids and need to be stabilized with antioxidants quickly after extraction. Another challenge is the rapid spoilage of marine raw material. In order to obtain high quality oil, the raw material supply chain must be carefully designed to avoid processing delays.

4.1.5 Cod liver oil

A mentioned above, cod livers are the primary storage site for body fat. As a natural cycle of food availability and roe maturation, the livers contain varying levels of fat and moisture throughout the seasons. During the late fishing season, from September to December in Newfoundland, Canada, the livers are at their fattest, containing ~50% fat, 30% moisture and ~20% protein (Table 2) (Mello and Rose, 2005).

Livers are enzymatic factories and contain high levels of active digestive enzymes: Lipases to break down lipids (fats), proteases to break down proteins and amylases to break down sugars and starches. After the fish dies, these enzymes continue to be active for hours to days, causing a fast break down of the liver tissue and lipids contained within (US Fish and Wildlife Service, 1948).

The natural break down of liver tissue has been utilized in the past to produce cod liver oil. In the traditional rendering process, a fermentation, the livers are placed in a barrel and the lid is closed airtight, with a one-way valve to let internally formed gases escape. Within days to a couple of weeks (temperature-dependent), liver enzymes break down the tissue and release the oil. The absence of air somewhat slows oxidative degradation. The released oil floats to the top while solids sink to the bottom

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and the water layer in between acts as a barrier. Without agitating the barrel, the oil is scooped off the top of the barrel and bottled (US Fish and Wildlife Service, 1948).

While fermented cod liver oil has had an important impact on the health of the people, providing important nutrients, fats and vitamins, generations all over the World have unpleasant memories to cod liver oil, remember "the spoon" they had to take daily as children, or remember the recurring burps the browned oil would cause for hours after taking it (Griffing, 2008; Banoub, 2018).

Today we know that this is because of the products of oil hydrolysis and oxidation. Volatile compounds like aldehydes and ketones evoke a repulsing sensation in our bodies – a protection mechanism that prevents us from eating spoiled foods. While the off-flavor and associated "fishy burps" are unpleasant, no significant adverse health effects have been associated with consuming fermented cod liver oil (Banoub, 2018).

Today most cod liver oil is produced using a gentle heat treatment under vacuum or a modified, oxygen-free atmosphere, yielding a lightly colored oil. Refining further removes any traces of fishy taste or smell which many consumers dislike. Unfortunately, because several rendering steps are performed at high temperatures, the nutritional quality suffers and fish oils are often supplemented with synthetic vitamins and omega-3 fatty acids after rendering (Vaisali *et al.*, 2015).

Liver freshness is known to be the single most important factor in the production of high-quality cod liver oil (Aas, Kjerstad and Barnung, 2016). It is therefore essential to ensure immediate and complete bleeding of the fish and to extract the cod liver oil as soon as possible after harvest. Ideally, the livers must be cleaned of foreign tissue and the gall bladder immediately after gutting. Ideally, the livers should be vacuum-packed and frozen. If this is not possible, livers shall be bagged with little air and placed on ice and out of sunlight right away to lower the temperature as fast as possible. Oil extraction should begin within two days of harvest, before tissue break-down and natural rendering (oil release) sets in (Karlsdottir *et al.*, 2016). The faster the oil can be removed from the active enzymes contained within the liver, the better. Even after extraction, the oil continues to be vulnerable to oxidative damage until it is stabilized by the addition of antioxidants.

The Newfoundland cod fishing industry presently requires fishermen to gut at sea right after harvest and the guts are disposed at sea. The collection of cod livers and the processing and cooling chain is currently not established and present a road-block in the implementation prospects of a cod liver oil extraction facility.

The client in this project is the operator of a cod ranch in Newfoundland. Ensuring an optimal cod liver collection in a ranch setting is much easier because a controlled number of cod are harvested at a time. Because gutting takes place on shore, the livers can be carefully collected, cleaned, and further processed. Excellent freshness can be ensured at every harvest from the cages.

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4.2 Degradation of oil

Degradation of oil is complex and occurs through several different pathways that produce many different chemical compounds as reaction products. In short, we differentiate oxidation, hydrolysis, and enzymatic degradation. Degradation is generally accelerated by light, heat, and the presence of oxygen. Fats and oils with a high degree of unsaturation (fish oils) are particularly sensitive to oxidative degradation, whereas saturated fats (coconut oil, palm oil, tallow) can easily withstand long storage and exposure to heat, light, and air. Products of oxidation are peroxides, aldehydes and ketones. Hydrolysis is the breakdown of lipids in the presence of water, accelerated by heat. The most prominent reaction product of hydrolysis are free fatty acids – they increase the acidity of oil. Enzymatic degradation occurs when internal enzymes are present or when the oil is exposed to microorganisms that produce enzymes. There are many different reaction products, including FFA's. Enzymatic oil degradation can be slowed by cooling, freezing, and taking away air and light exposure but it will continue until the enzyme is denatured/inactivated or separated from the oil.

Literature specific to cod liver degradation is limited. Karlsdottir (Karlsdottir *et al.*, 2016) investigated lipid degradation of cod liver during frozen storage and found vacuum packaging much superior to simple plastic bagging. Lowering the temperature as much as possible makes a big difference in slowing lipid degradation, even if the difference is just 6 °C. Finally, they pointed out that the increase in FFA levels is independent from the packing method because the mechanism is enzyme-driven and does not rely on the presence of oxygen, light or heat.

Hansen-Aas published a study on quality and shelf life of refrigerated farmed cod liver. (Aas, Kjerstad and Barnung, 2016) They placed strong emphasis on the gentle on-board handling, fast bleeding, careful and complete cleaning, immediately followed by chilled storage of the liver to ensure best quality. Importantly, they found that sensory analysis (smell and tissue firmness) of the liver tissue usually detects the first signs of spoilage after 3-4 days of chilled storage (slight rancid odor and softening of tissue), even before chemical analysis can provide clear results. They recommend that canning occurs within 2 days of harvest to prevent oil leaking from the tissue. Table 1 shows their grading system for fresh cod liver.

***Table 1: Quality evaluation of fresh cod liver* (Aas, Kjerstad and Barnung, 2016)**

Quality parameter	**Rating**		
	Consumption 9-7	**Processing 4-6**	**Discard 1-3**
Color	light beige, barely greenish	some green discoloration	Greenish throughout the organ
Odor	Sea-fresh, low intensity	slightly rancid	strong rancid odor
Texture	Firm, good elasticity	soft	partially disintegrated
Oil leakage	No visible oil leakage	<50% oil leakage	>50% visible oil leakage
Blood spots and bruising	Not observed	some blood spots	a lot of blood spots and discoloration
Liver processing	Liver only	some appendage of intestine	Intestine appended to the liver
Overall impression	Superior	Good	Rejected for consumption

In conclusion:

- Fish must be bled immediately after harvest.
- Livers must be immediately cleaned and placed on ice, away from sunlight and heat.
- Further processing of livers (freezing or oil extraction) must commence within 2 days of harvest
- Oil extraction from frozen livers must be completed within 1 month from harvest.
- A stabilizer/antioxidant must be added to the oil immediately after extraction.

4.3 Cold-extraction of fish oil

Literature is very limited when it comes to cold extraction of fish oil. The demand for extra-virgin cod liver oil is just beginning to grow and companies that have developed a process have not published it. There is, however, information on the cold extraction of other edible oils, from which a process can be designed.

To our current knowledge, there is only one extra virgin cod liver oil product on the market, which his called Rosita. While the process is proprietary, some clues are given by the company that give us some indications about their process (https://www.rositarealfoods.com/general-information/42-the-rosita-method):

- The process, from fishing to bottling, is completed within 48 hours at room temperature or below, and only fresh livers are used.
- Cod livers are cut with ceramic knives to avoid oxidation and livers do not come into contact with metal throughout the process.
- A natural antioxidant (organic rosemary herb and organic vitamin E) is added to the oil to prevent oxidation of the polyunsaturated fatty acids.
- No flavors or additives are used to disguise the natural taste
- The oil "bites a little" in the back of your throat.
- A decanter-style set-up is used to separate the oil form the tissue during rendering: The liquid drips into a vessel. The oil floating on top can overflow into another collection vessel while the water and protein sludge remain behind.
- Refining is kept to a minimum:
    - Charcoal filtration (absorption of some contaminants, e.g., heavy metals, POP)
    - Finishing filtration to remove suspended particles.
    - Proprietary "sponge-like" process that removes contaminants without the use of heat or solvents.
- Nutritional claims:
    - Vitamin A: 3000-5000 IU/teaspoon (slight seasonal variations)
    - Vitamin D3:400-500 IU/per teaspoon (slight seasonal variations)
    - A to D ratio of 10:1

The following paragraphs summarize publications on fat cell lysis methods for edible oil extraction that do not apply heat.

Zinnai (Zinnai *et al.*, 2015) presented a cryo-assisted extraction process for Extra Virgin Olive Oil (EVOO). By mixing solid CO_2 (carbonic snow) directly while grinding the olives cause water in the cells to freeze and expand beyond the capability of the cell walls to contain. After grinding, a layer of cold CO_2(gas) remains layered over the paste, slowing oxidative degradation. This process significantly increased the oil yield and the accumulation of tocopherols in the liquid phase.

(Trilaksani *et al.*, 2020) extracted virgin tuna oil from tuna eyes by freeze/thaw, followed by crushing and centrifugation. They did not, however, provide yield numbers and also noted that they observed significant degradation during cold-storage (-20°C) of the raw material.

Głowacz-Różyńska (Głowacz-Różyńska *et al.*, 2016) developed a cold-extraction method for salmon salar skins and heads which resulted in similar yields (95% for skins, 71% for heads) as hot extraction and enzymatic extraction methods but the extracted oil had lower FFA and oxidation values. By-product was ground through 5mm blade (benchtop meat grinder), then frozen in 250 g portions. For extraction, the frozen (or partially frozen) raw material was mixed with 50°C hot water (1:1 w/v) and blended with a kitchen hand blender for 5 min. The temperature never exceeded 15°C. Then the paste was centrifuged at 8000 x g for 10 min, decanted and centrifuged again.

Next to applying freeze/thaw cycles, ultrasonic extraction was used in several publications. The treatment uses mechanisms of sound waves travelling through a solution, causing alternating high and low pressures in the solution. During the low-pressure stage, millions of microscopic bubbles form and grow. This process is called cavitation, meaning "formation of cavities". During the high-pressure stage, the bubbles collapse, or "implode", causing high shear forces that can rupture the membranes of cells (Abdullah *et al.*, 2010). All publications have in common that they all dilute the raw material with a solvent during sonication (Abdullah *et al.*, 2010; C. H. Kuo *et al.*, 2017; Rosas-Mendoza *et al.*, 2017).

(Rosas-Mendoza *et al.*, 2017) performed a study on cold extraction of oil from chia seeds. They used a combination of cryogenic grinding (using an impact knife and liquid N2), followed by dilution with ether and ultrasonic bath treatment (40 KHz), resulting in 79.3% oil yield. They conclude recommending the use of an ultrasonic probe instead of a sonicator bath to promote energy efficiency and uniform energy distribution.

Another example of ultrasonic- assisted oil extraction from eel was presented by Abdullah (Abdullah *et al.*, 2010). After producing a powder by drying and grinding, they diluted the powder with Ethanol and placed the sample in sonicator bath for 60 min at room temperature (25 kHz, 200W). Afterwards the solids were filtered out and the ethanol was evaporated. It was found that higher amounts of solvent and longer sonication increase the yield but found a decrease in yield if sonication strength exceeded 200W.

Kuo (C. Kuo *et al.*, 2017) extracted oil from fish liver (Cobia) and compared different extraction methods, all based on the solubilization of the oil by Hexane following a homogenization step. The best yields were obtained using polytron homogenization and a 1:10 oil/hexane mixture. Sonication of the mixture in a Branson ultrasonic bath 2510, 40 kHz for 15-120 min further increased the oil yield. Confirming observations by Abdullah, they found higher yields when increasing the amount of solvent. No improvement was seen when the polytron speed was increased.

In conclusion:

- Sonication treatment has increased yields in multiple studies.
- Most cold extraction processes from oil seeds employ solvents.
- The destructive effects of thermal lysis (freeze/thaw cycles) on fish tissue have been shown in multiple studies (Islam, Aryasomayajula and Selvaganapathy, 2017)
- Osmotic shock may be suitable for fish cells as the cell membranes are delicate and rupture easily. (Islam, Aryasomayajula and Selvaganapathy, 2017)

4.4 Industrial refining of edible oils

Over the past decades, edible oil refining has developed into a sophisticated industry, able to remove almost any unwanted components from an oil: FFA, phospholipids, oxidation products, heavy metals, POP's, color, odors, etc.

The international industry is dominated by a few large companies. To allow cost optimization, plants are only considered viable if exceeding a threshold of 4000 tonnes/day and set-up as a mono-feedstock establishment (Hamm, Hamilton and Calliauw, 2013).

Edible oil refining includes a series of steps, some based on chemical stripping of a component, others based on absorption, and yet others based on the specific removal of components by partial vacuum distillation.

When undergoing a full refining process, many oils obtain a bland taste and become nutritionally damaged. While sometimes a bland, neutral oil is desired, over-refined oils rely on the addition of synthetic omega 3 fatty acids and vitamins to reach their advertised nutritional potential.

While customers used to prefer fish oils that did in no way taste or smell like their organism of origin, growing importance of nutritional quality, environmental considerations, and sustainability aspects (additives, processing aids) have been drivers for new approaches to refining.

4.4.1 Degumming

Degumming is usually the first step in refining and is performed for 3 reasons:

- To produce lecithin (phosphatides) as a by-product
- To provide degummed oil for long-term storage or transport
- To prepare the oil for the following refining steps (Lecithin and other phospholipids are emulsifiers that decrease yields in downstream refining steps).

However, in oil that only requires little refining, the preferred choice may be to leave phospholipids in the oil as they possess multiple health benefits (Küllenberg *et al.*, 2012).

Degumming is usually performed by mixing the oil with acid at elevated temperatures, which dissolves phospholipids in the water phase which is subsequently removed.

4.4.2 Neutralization

Following degumming, edible oil is usually neutralized with a base at elevated temperatures. This is removes free fatty acids and acid traces left in the oil after degumming. Fish oil of exceptional freshness, however, should have very low free fatty acid levels, making this step unnecessary.

4.4.3 Bleaching

Bleaching is usually achieved by heating the oil to about 100°C and passing through a bed of activated earth, activated carbon, or amorphous silica. [2] The small amounts of adsorbent carried along by oil are removed by filtration.

Bleaching is basically an adsorption process that removes not only color compounds but also other minor impurities. The residual soaps are removed, and peroxides are decomposed into aldehydes and ketones due to further oxidation. These decomposition products are also adsorbed to the bleaching agent. Bleaching also removes a portion of POP's and heavy metals.

Thereby stability and flavor of the oil are also improved. Therefore, the TOTOX value (the sum of the p-anisidine value and twice the peroxide value) is used as one of the parameters to evaluate the bleaching operation (Vaisali *et al.*, 2015).

4.4.4 Deodorization

Deodorization nowadays is a multi-step process vacuum distillation at very high temperatures. The following is achieved:

- Further removal of FFA
- Thermal bleaching of pigments
- Removal of valuable compounds like tocopherols & sterols
- Removal of contaminants like pesticides and polycyclic aromatic hydrocarbons

Negative side effects are formation of trans fats and poly TG's. Depending on oil composition, time, temp, pressure, and steam can be fine-tuned to optimize outcome for the specific oil. Vacuum distillation requires a high initial investment and commercial plants do not operate below 1500 tonnes/day (Hamm, Hamilton and Calliauw, 2013; Vaisali *et al.*, 2015).

4.4.5 Winterization

Dewaxing or winterization is one of the oldest refining procedure and traditionally chemical-free. Originally, winterization was used to give the oil a good appearance during storage as it removes the oil components that settle to the bottom of the bottle during storage at cool temperatures.

After extraction, the oil is placed in a chill room for several days where it cools down slowly, allowing waxes and long-chained, saturated fatty acids to crystallize. Centrifugation can quickly and efficiently remove those crystals, leaving an oil that has much enhanced storage properties.

In fact, winterization is a simple method to concentrate omega-3 fatty acids by removing some other components from the oil (Méndez and Concha, 2018) .

4.5 Membrane-based Oil Refining

Modern materials science has encouraged researchers to design filtration mediums with a multitude of characteristics in terms of electrostatic charges, pore sizes, adsorptive and absorptive properties, and specificities to certain compounds. These developments have led to increased research activities over the past decade into the physical refining of edible oils based on membrane technology. Ultimately, the goal of this research is to find refining solutions that can compete with and replace the traditional, refining methods described above.

We performed a literature review of recent developments in the field of membrane-based refining of edible oils. For further reading, we recommend the review by Manjula and Subramanian 2007 as an excellent introduction to membrane technology for refining of edible oils (Manjula and Subramanian, 2006).

To understand oil refining, we must first look at the general composition of an oil prior to refining. We will use a figure from the above mentioned review paper to show the oil components on a scale of molecular weight. The figure was developed for vegetable oils - this distribution is slightly different for marine oils as the average MW of the fatty acids is higher, but the idea is the same.

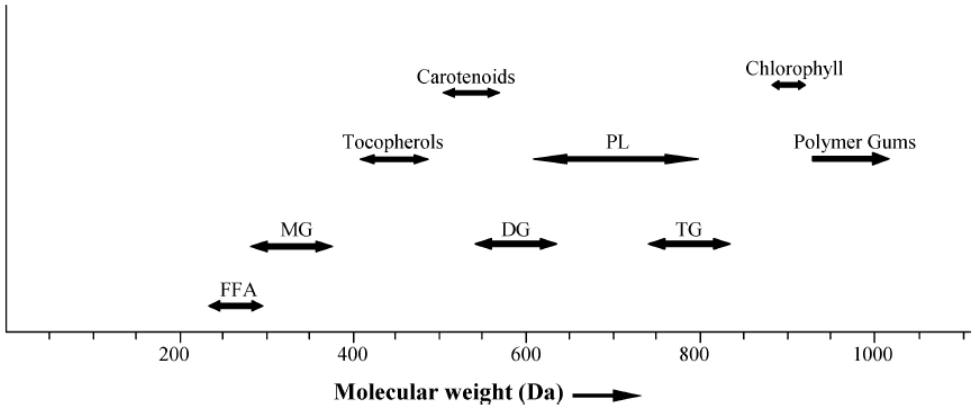

Figure 4: Molecular size distribution of oil constituents **(Manjula and Subramanian, 2006)**

The basic idea of membrane filtration is straight forward, it its' simplest form, smaller compounds pass through the membrane while larger ones are retained. By choosing the molecular weight cut off carefully, we can separate wanted and unwanted compounds. The bigger the size difference between the compounds, the better the separation.

The separation of oil components is more complex than that because oil is a mixture of fatty acids of different lengths and other compounds of varying molecular size. For example, fatty acids can be present as individual fatty acid chains (free fatty acids) or can be bound to a glyceride molecule, together with up

to three other fatty acids (mono-, di-, or triglycerides) – all of which have different molecular sizes and may or may not be desired for the product. Phospholipids (usually unwanted) span a wide range of molecular weights and show overlap with triglycerides (wanted). Such overlap requires the use of membranes that separate molecules not only based on their size but based on charge, affinities, 3D structure or other characteristics.

Some very small components of the oil like FFA, monoglycerides, or tocopherols require very small pore sizes or diffusion membranes (nanofiltration, or reverse osmosis membranes) which are chronically prone to clogging and provide very slow flow rates at best (de Morais Coutinho *et al.*, 2009; Tres *et al.*, 2010; Vaisali *et al.*, 2015).

On the other hand, the removal of particles larger than TG has been found to be more promising. For example, waxes and polymer gums can be efficiently removed using microfiltration membranes with mean pore diameters from 0.05 to 1 µm (de Morais Coutinho *et al.*, 2009).

While edible oils are liquid at room temperature, they are viscous liquids and do not easily flow through a fine, semi-permeable membrane. This is often described as the main problem in membrane refining of edible oils and is usually addressed by the addition of a solvent to improve flow characteristics of the oil during refining.

4.5.1 Membrane types

The following general definitions of membranes was taken from the Snyder Filtration webpage:

https://synderfiltration.com/learning-center/articles/introduction-to-membranes/polymeric-membranes-porous-non-porous/

Porous and polymeric membranes have a thin layer of semi-permeable material that is used for solute separation as transmembrane pressure is applied across the membrane. The degree of selectivity is largely based on the membrane charge and porosity. Membranes with symmetric pores are more uniform, while asymmetric pores have variable pore diameters.

Porous

Porous membranes are mainly used for microfiltration and ultrafiltration. The membrane contains pores ranging from 0.1 to 10 µm for microfiltration and 0.001 to 0.1µm for ultrafiltration. The separation is based on particle size. In order to achieve high selectivity, pores on the membrane need to be relatively smaller than the particles in the mixture. One disadvantage of using a porous membrane is membrane fouling that causes flux decline over time. Chemical and thermal stability are also significant factors to consider when selecting porous materials because temperature and concentration affects selectivity and flux of the membrane.

Non-porous

Non-porous membranes are mainly used for reverse osmosis, nanofiltration, or molecular separation in the gas phase. The membrane is a dense film where permeate diffuse through by pressure, concentration,

or electrical potential gradient. The polymeric material affects the permeability and selectivity of the membrane. The separation process occurs from differences in solubility and diffusivity. One disadvantage of non-porous membranes is low flux; therefore, the dense film is usually made extremely thin and is deposited on top of asymmetric membranes.

4.5.2 Membrane Degumming

Of all refining steps, degumming using membrane technology has received the most attention. Research has been conducted into degumming of diluted and undiluted oils.

4.5.2.1 Membrane degumming of undiluted oils

As shown in Figure 4, PL and TG overlap in their MW and are therefore not well suited for efficient separation by porous membranes. For degumming of undiluted oils, a non-porous membrane is needed (i.e. nanofiltration, RO) where separation is not based on pore size but happens due to solution-diffusion effects. Filtration through such fine membranes, in combination with the high viscosity of undiluted oils, results in extremely low flow rates across the membranes and accentuated fouling (Pagliero *et al.*, 2001; de Morais Coutinho *et al.*, 2009).

Only one publication was found that investigated degumming of undiluted oils. NTGS 1100 and NTGS 2100 membranes have been successfully used for degumming of undiluted oils in batch stirred cell reactor (Manjula and Subramanian, 2009). These membranes are polymeric membranes with silicone as the active layer and polyimide and polysulfone as support layers (Nitto Denko Corporation, Kusatsu, Japan), originally developed for gas-separation applications and used for hexane recovery in the petroleum industry. The filtration was performed at room temperature. Stirring at 800 rpm during filtration was performed "to minimize concentration polarization effect".

The removal of phospholipids reached above 99% for undiluted oil using both membranes, but the oil flux was too low (0.03 kg/m^2 h), and they concluded that membrane degumming of undiluted oils does not have promising commercial applications.

4.5.2.2 Membrane degumming of diluted oils

To solve the problem of overlapping molecular size of phospholipids and triglycerides, researchers have made use of the ability of phospholipids to form micelles.

Having a hydrophilic head and a hydrophobic tail, phospholipids arrange themselves in micelles when dissolved in aqueous solution - with the hydrophilic head pointing outwards. When diluted with a polar solvent, usually hexane, phospholipids form **reverse micelles**, where the hydrophobic fatty acids point outward and the hydrophilic head inwards.

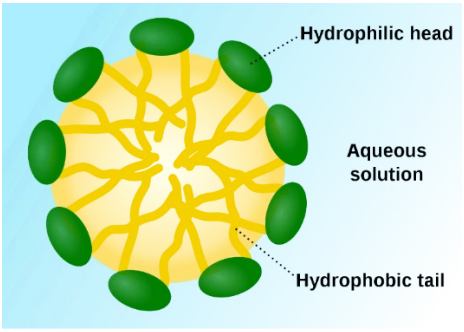

Figure 5: Phospholipid micelle in aqueous solution (https://en.wikipedia.org/wiki/Micelle)

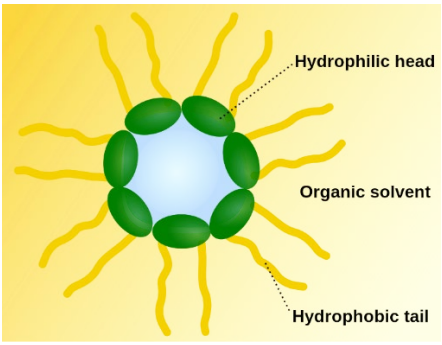

Figure 6: Phospholipid micelle in non-polar solution (https://en.wikipedia.org/wiki/Micelle)

Depending on the phospholipid concentration and hexane/oil ratio, these micelles are much larger (18-200 nm or ~500 kDa) than triglycerides and can be filtered out easily using porous filtration membranes (MWCO: 20 kDa) used in micro and ultrafiltration. This is referred to as "Micelle-Enhanced Ultrafiltration" or MEUF. The flow through the membranes is faster by one order of magnitude and filtration can be conducted at low temperatures. (Gupta, Muralidhara and Davis, 2001). Badan and Ribeiro also successfully investigated the soybean oil degumming on a pilot plant scale using a ceramic membrane (Badan Ribeiro *et al.*, 2008).

The "reverse micelle" approach is very suitable for degumming of vegetable oils that are obtained through solvent extraction and therefore already mixed with hexane. There, MEUF does not increase chemical use. Hexane can be recovered and recirculated with relative ease in an industrial setting. In addition, micelles encapsulate other contaminants in their center like copper, iron, magnesium and calcium.

The challenge with MEUF is that hexane often affects the filtration polymer, causing swelling and a reduction of flux. Choice of filtration material is important.

Fish oils are usually not extracted using a solvent. Therefore, degumming using micelle technology would mean the addition of a volatile chemical to the process which requires the installation of a specialized processing equipment and hexane-recovery system. This would come at a significant additional cost, making it cost-prohibitive.

4.5.3 Neutralization (FFA removal)

No breakthrough has been achieved for membrane-based removal of FFA and the chemical refining process requires the addition of alkali. Some FFA can be removed by filtration as the MW is smaller than Triglycerides but removal is not highly efficient (Manjula and Subramanian, 2006).

Extracting cod liver oil from properly handled livers of exceptional freshness, the FFA content should be naturally low and a FFA removal step should not be required.

4.5.4 Bleaching

No breakthrough has been achieved for membrane bleaching of undiluted, unheated oils. Depending on the environmental contamination levels and oxidative quality of the oil, a bleaching step may not be necessary.

One study was found on bleaching at room temperature: Huang and Sathivel investigated the removal of peroxides and FFA from salmon oil using chitosan or activated earth at room temperature. The found that activated earth removed more peroxides than chitosan but neither of the two was effective for FFA's. Both adsorbents effectively increased the lightness and reduced the redness of the oil. The activated earth also had a good ability to adsorb peroxides, minerals, moistures, and insoluble impurities of unpurified salmon oil (Huang and Sathivel, 2010).

4.5.5 Deodorization/POP removal

Ortiz studied the elimination of persistent organic pollutants (POP's) from fish oil with solid adsorbents (Ortiz *et al.*, 2011). Fish oil can contain high concentrations of persistent organic pollutants due to their lipophilic properties. This study investigated the effect of eleven silicon-based and nine carbon-based adsorbents on the removal of organic pollutants, including polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB) or dichloro-diphenyl-trichloroethane (DDT) and polybrominated diphenylethers (PBDEs).

No significant elimination was observed with any of the silicon-based adsorbents studied. For carbon-based adsorbents, PCDD/Fs removals from fish oil with activated carbon were very high and moderate for dioxin-like PCB's and HCB, DDT, and PBDE. Adsorbent amount was the most influential factor in the POPs removal process. Optimized adsorption conditions were with 2.5% of adsorbent amount during 37.5 min of adsorption time at 1000 hPa and 80 °C, using activated carbon (coconut shell) as adsorbent and refined salmon oil. With these experimental conditions, obtained removals were: 99% PCDD/F, 70% HCB, 36% dioxin-like PCBs, 27% DDTs, 11% marker PCBs and 9% PBDEs. The fatty acid profile was analyzed for the oil after treatment, and there was no significant change observed (Ortiz *et al.*, 2011).

4.5.6 Winterization

Dewaxing is traditionally done by cooling the oils slowly and then removing the crystallized waxes by centrifugation or filtration, for example through a 120 nm HDPE membrane in a cross-flow setting (Manjula and Subramanian, 2006). A disadvantage of membrane dewaxing is that the waxes clog the membrane easily and a back wash protocol has to be developed to maximize membrane life. For example,

back wash with hot water can be used periodically to remove wax build-up. It has been found that rapid cooling followed by a maturation period works just as well as a slow cooling procedure. The publication referred to "crash cooling" + maturation (Méndez and Concha, 2018).

Centrifugation appears the superior choice of methods for winterization as it completely avoids membrane fouling and the accost associated with replacing the membranes.

In conclusion:

- In spite of intensive research, a breakthrough for membrane refining of undiluted oils at room temperature has not been achieved
- Some refining steps do show potential to be replaced by membrane technology
    - Degumming using MEUF technology
        - Mostly suitable for solvent-extracted vegetable oil
        - Not suitable for fish oil
    - Winterization
        - Crystals that formed during cooling of the oil can be removed by filtration.
        - However, centrifugation is easier and prevents membrane clogging
    - Deodorization (POP removal)
        - Carbon-based absorptive filtration medium
        - Heating of the oil is needed (80°C)

4.6 Raw material quality and composition

In total, 4 samples of cod livers were received. Sample #1 was received in late Dec 2019 and Covid-19 lockdown delayed the project start until June 2020. At that time, the sample was spoiled and unusable for the purpose of this study (see Figure 8). Shipments #2 and #3 were of excellent quality and provided in sufficient amounts to allow for several experiments as well as moisture and lipid analysis. The majority of experiments was performed with those two shipments. The results are shown in Table 2. Sample #4 was collected towards the end of the fishing season and upon receipt, the livers were slightly dried out and a little (1 day) older than samples #2 and #3. Sample #4 was not sufficiently large to perform moisture and lipid analysis.

Table 2: Moisture and lipid analysis of cod livers

Sample	Quality Grading of fresh samples**	Moisture	Lipids (dry basis)	Lipids (wet basis)*
Sample 1, Dec 2019	7 (fresh sample) 1 (after 6 months frozen storage)	n.d.	n.d.	n.d.
Sample 2, Nov 2020	9	35.45 % ± 0.17	85.70 % ± 0.38	56.18 %
Sample 3, Dec 2020	9	36.53 % ± 0.07	84.35 % ± 0.58	53.54 %
Sample 4, Jan 2021	7	n.d.	n.d.	n.d.

**by calculation, **based on Table 2*

In rendering experiments using sample #4 livers, the average lipid content obtained for samples #2 and #3 were used to calculate oil extraction yield.

4.7 Rendering experiments

Eleven rendering experiments were performed in total. Table 3 details experimental conditions and observations and the results are discussed in detail in the following paragraphs. The highest oil yield and best oil quality (by color, clarity and smell of the oil) was achieved in experiments #2 and #9 using the following process:

- **Chopping with plastic knife (1 cm pieces)**
- **Vacuum packaging (thin bags)**
- **Frozen storage (-20°C) for 7 days**
- **Thawing in sonicator bath (40 kHz, 30 min at 20°C)**
- **Rendering under vacuum (-20" Hg) in flat mesh & china cap filter set-up (Figure 2) for 24 h**
- **Phase separation in separatory funnel, collection and cooling of oil to 2-8 °C overnight**
- **Centrifugation of cooled oil for 40 min at 10,000 rpm**
- **Flushing of oil with Nitrogen and storage of oil at -80°C until analysis**

The recovered oil of those experiments is shown next to the Rosita™ market sample in Figure 7. Detailed analysis was only performed on those two samples due to budget constraints.

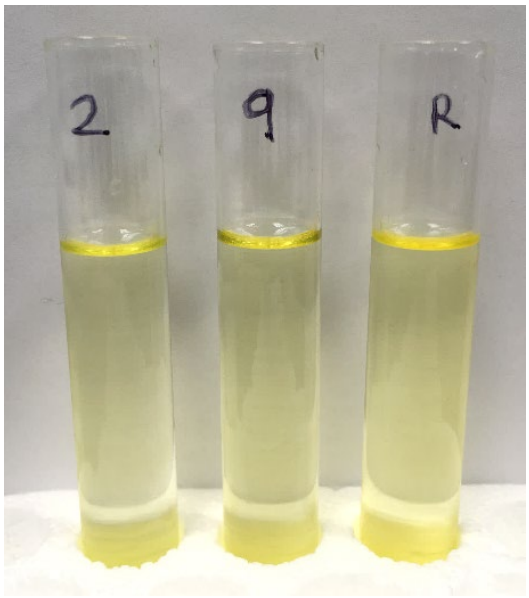

Figure 7: Recovered & winterized oil from Exp 2 and 9 next to Rosita™ oil "R"

Table 3: Experimental data - cold vacuum rendering

	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8	Exp 9	Exp 10	Exp 11
Date	*Dec 1-2*	*Dec 2-3*	*Dec 3-4*	*Dec 4-5*	*Dec 7-8*	*Dec 8-9*	*Dec 9-10*	*Dec 10-14*	*Dec 15-16*	Jan 14-15	Jan 21-22
Raw material sample	#2	#2	#2	#2	#3	#3	#3	#3	#3	#4	#4
Days frozen	7	8	9	10	7	8	9	10	14	never frozen	7
raw material (g)	1000	1000	1000	1000	780	779	1000	1000	1000	1000	1000
dry weight (g)	655.51	655.51	655.51	655.51	511.30	510.65	655.51	655.51	655.51	645.10	645.10
fat (g)	561.76	561.76	561.76	561.76	431.28	430.73	552.92	552.92	552.92	548.48	548.48
Treatment	whole, vacuum	chopped, vacuum, sonicated	chopped, vacuum, decanter set-up	whole, no vacuum	ground, vacuum, sonicated in thick bag	ground, vacuum	chopped, vacuum, sonicated in thick bag	Whole, vacuum, sonicated in thick bag	chopped, sonicated 500 g at a time (thin bags)	chopped, sonicated 500g at a time, thin bags	whole, sonicated 500 g at a time in thin bags
Experimental set-up (see Figure 2)	A	A	B	A	A	A	C	C	C	C	C
total liquid recovered (ml)	20 (6h) 650 (24h)	590 (17h) 700 (24h)	455 (17h) 545 (24h)	550	500	515	650 (24h)	490 (21h) 560 (24h) 685 (4d, rancid)	450 (4h) 640 (21h) 685 (24h)		
recovered oil (ml)	350	450	295	280	250	305	345	350	440	24	200
recovered oil (g) (based on a density of 0.93 g/ml)	325.5	418.5	274.35	260.4	232.5	283.65	320.85	325.5	409.2	22.32	186
% recovery	**57.94**	**74.50**	**48.84**	**46.35**	**53.91**	**65.85**	**58.03**	**58.87**	**74.01**	**4.07**	**33.91**
Smell (1 = none, 2/3 = fishy, 4 = unpleasant fishy to 5 = rancid, putrid)	1	1	1	2	3	3	1	1 (5 after 4 days)	1	3	2
Oil drainage (1=excellent draining, 2=small oil pockets, 3=large oil pockets)	2	2	3	3	2	2	2	3	2	3	2
Phase separation (1=very good to 3=bad)	3	1	1	1	3	1	1	1	1	2	3
Sludge (1=no sludge, to 3=lots of sludge/clogging of filter	3	1	after 17 h: 1 after 24 h: 2	2	3	1	1	2	1	2	
Oil color and appearance	light yellow, no smell, cloudy	light yellow, green hue, cloudy	light yellow, cloudy	dark yellow, cloudy	dark yellow cloudy	dark yellow/light brown, cloudy	light yellow, cloudy	light yellow, green hue, minimal cloudiness	light yellow, cloudy	dark yellow/light brown, cloudy	
Notes	almost no draining in the first 6 h	significant liquid release after sonication	Oil collected in overflow: crystal clear light yellow,		significant liquid release after sonication	very dark, bloody water phase	rendering not complete after 24 h	sonication hindered by thick vacuum bag	fine sludge clogging china filter - oil can't drain and becomes rancid	raw material not great	

4.7.1 Effect of particle size reduction

Cod liver tissue breaks down quickly when compared to other body tissues (within hours to days, depending on freshness and temperature), even without a tissue-disrupting treatment. If the liver cell membranes are weakened by cryo-treatment and/or sonication, tissue breakdown and the associated oil release can be accelerated. Particle size reduction can improve drainage of the released oil.

In this study we compared the effects that grinding or chopping has on oil draining compared to rendering whole livers. We found that chopping prevented the formation of large oil pockets in the tissue, thereby improving oil drainage significantly when compared to whole livers. However, not all released oil could drain and collected in smaller oil pockets throughout the tissue.

Grinding resulted in good drainage of the oil overall, however, a lot of fine sludge was produced, which negatively impacted phase separation and oil quality. For example, in experiment 5 (ground and sonicated), the tissue damage was severe, leading to the formation of a lot of fine sludge that clogged the filter and in turn hampered oil collection (Figure 10, Figure 13). In experiment 6 (ground, not sonicated), a relatively high oil yield was achieved (70.81%) but the oil was brown, leading to the conclusion that the oil degradation was caused by grinding of the livers.

The mechanism for this spoilage is unclear. A number of factors should be considered:

- A lot of tissue surface is exposed to air during grinding when compared to chopping livers or rendering them whole. This may accelerate oxidative spoilage
- The tissue comes into contact with metal, which can promote auto-oxidation (Choe and Min, 2006). If this is the cause, grinding with plastic equipment may remedy the problem. This could be investigated in a phase 2 study.
- Grinding may increase exposure of the oil to internal enzymes

In conclusion, chopping of livers is considered to be the best option to improve oil drainage without producing fine sludge. However, drainage must be further improved by investigating rendering set-up and gentle tissue agitation.

4.7.2 Effect of freezing

As expected, the freeze/thaw cycle resulted in significant tissue damage and dramatically increased the oil release. To confirm its strong effect, one experiment was conducted with fresh, never frozen livers (Exp 10- never frozen, chopped and sonicated), resulting in an oil yield of only 4.38% within the usual 24 hr rendering period. In all other experiments, livers were frozen before rendering.

It was observed that the duration of frozen storage significantly influences tissue damage and oil release, confirming, published studies (Aas, Kjerstad and Barnung, 2016; Karlsdottir *et al.*, 2016). Figure 8 and Figure 9 illustrate the changes occurring during frozen storage of cod livers. Fat cells in a sample that was stored frozen for one day were intact after thawing and no oil was released. After 7-days storage, tissue damage was clearly visible upon thawing and some oil was released. The oil had a yellow color and

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pleasant smell. After 6 months' storage, the tissue was completely disintegrated and the oil was brown and rancid (Figure 8-B).

Based on this preliminary data, we conclude that frozen cod liver oil deteriorates to levels unacceptable for extra-virgin cod liver oil within about 3 months of frozen storage. However, a few days of storage appears necessary for ice crystals to grow and the desired tissue damage to occur. Extraction of the oil is therefore recommended to take place between 1 and 4 weeks of frozen storage.

We recommend that the optimal frozen storage length is determined in a follow-up study.

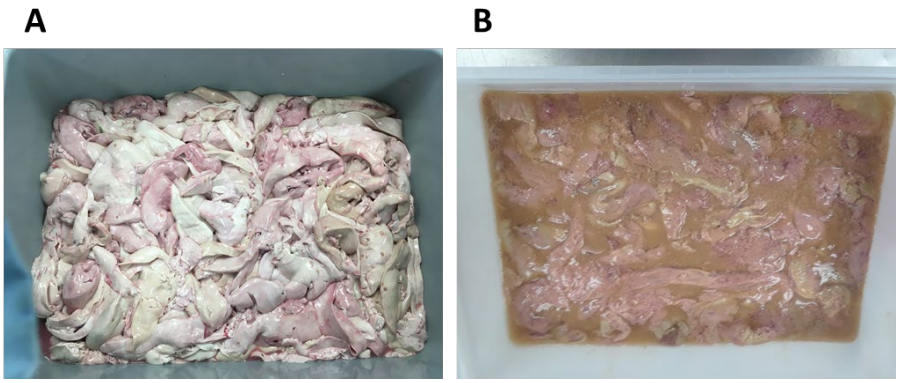

Figure 8: A - Appearance of fresh livers, e.g., SMP's 2 and 3; B- Livers thawed after 6 months frozen storage (spoiled)

Figure 9: Moisture analysis of blended raw material. SMP 1 (left) was frozen for 7 days while SMP 2 (right) was frozen for 1 day only – oil release is happening in the older sample while no oil separates from the 1-day old sample

4.7.3 Effect of sonication

Confirming other published studies, sonication increased tissue damage and oil release. In this study, a standard treatment of 30 min sonication at room temperature and 40 kHz was applied. The frozen vacuum pouch was placed into the sonicator water bath and thawed out during sonication treatment. After 30 min, the tissue was completely thawed and transferred to the rendering set-up.

Sonication caused the immediate release of significant amounts of oil and water, especially in chopped liver samples. Rendering occurred much faster than in un-sonicated samples.

Excessive sonication, or the combination of several tissue-weakening treatments (e.g. frozen storage, sonication and grinding) will disrupt the tissue too much, resulting in the formation of fine sludge, which clogs the filter and negatively affects phase separation. Examples of good and bad phase separation are shown in Figure 10.

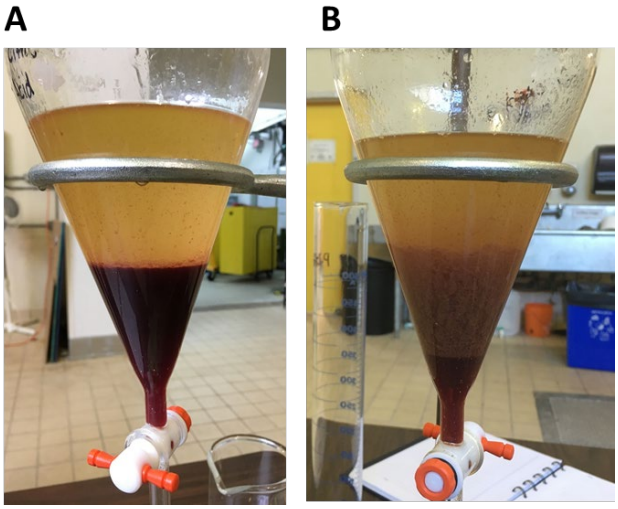

Figure 10: Example of good (A) and bad phase separation (B)

The application of sonication in this study had its limitations. The sonication set-up in this study was not ideal. The use of a sonicator water bath did not allow for even penetration of the sound waves into the vacuum-packaged tissue, Furthermore, sonication was hindered by the use of thicker vacuum bags (Exp 5, 7, and 8) and the capacity of the water bath was limited to one 500 g pouch at a time.

We recommend that the sonication procedure is optimized in a follow-up study, for example using a sonication probe, immersed into the liver tissue.

4.7.4 Rendering set-up

Vacuum and temperature. Rendering cod liver tissue under vacuum at 17°C was effective and provided oxygen-free rendering conditions that prevented oil oxidation. Because the effects of vacuum are well known, only one experiment was performed without vacuum (Exp 4) and compared to an identical experiment with vacuum (Exp 1). It confirmed that rendering in the presence of oxygen causes visible oil degradation (deterioration of oil color and smell) within the 24 h rendering time. While vacuum slows down spoilage, it does not prevent it. The rendering temperature is therefore important in controlling the speed of rendering – higher temperatures speed up both oil release but also oil degradation. The optimal temperature has to be determined. However, this was outside the scope of this study and the

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temperature was kept constant at 17°C. This temperature resulted in rendering of the majority of the oil, however, oil spoilage was only observed in experiments where rendering was observed over several days.

As was observed in Exp 8, if the tissue is left in the rendering set-up (under vacuum) for four days, the collected oil is spoiled (brown color and rancid smell). The good yield numbers obtained for Exp 2 and 9 (highest yields and best oil) indicate that 17°C is an appropriate temperature to allow the release of the majority of oil from the tissue within 24 h.

Fine tuning of rendering time and temperature could further increase final yields. For larger batches, the use of Nitrogen instead of vacuum might be easier to implement than a vacuum chamber but it must be confirmed that rendering under Nitrogen produces oil of similar quality.

Hanging 0.5 mm mesh. A soft, hanging 0.5 mm mesh provided gentle pressure to the tissue, supporting oil drainage. Furthermore, as the liquid was released over time, the tissue slowly moved further down into the mesh – thereby gently turning the tissue over and preventing the formation of oil bubbles (Figure 11). Fine sludge passes through the mesh. The mesh can be cleaned with ease and reused many times.

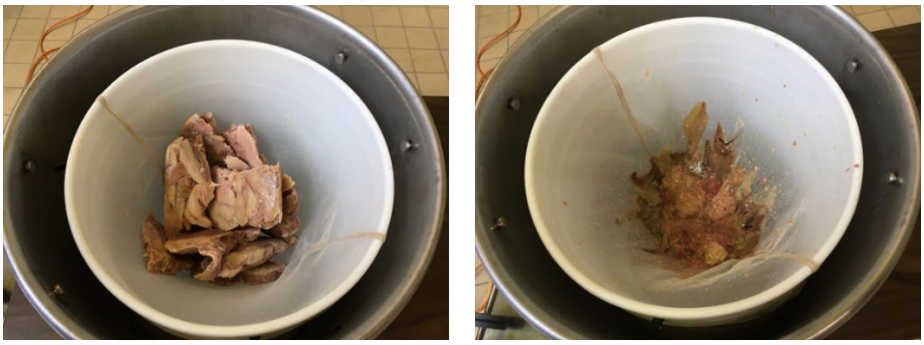

Figure 11: Experiment 1 - whole livers placed into mesh frozen (left) and after 24 hours of natural rendering under vacuum (right)

Flat 0.5 mm mesh. The flat mesh allowed the tissue to spread out over a larger horizontal area as it becomes soft. However, some oil collected in pockets (~1-3 mm diameter) on top of the tissue and did not drain (Figure 12). Furthermore, oil drops hung on the bottom of the mesh until they became too heavy and dripped into the collection flask. This delayed the separation of oil from tissue enzymes. Oil pockets were larger when whole livers were rendered compared to chopped liver. The mesh can be cleaned with ease and reused many times. In conclusion oil drainage was less efficient than in the hanging mesh.

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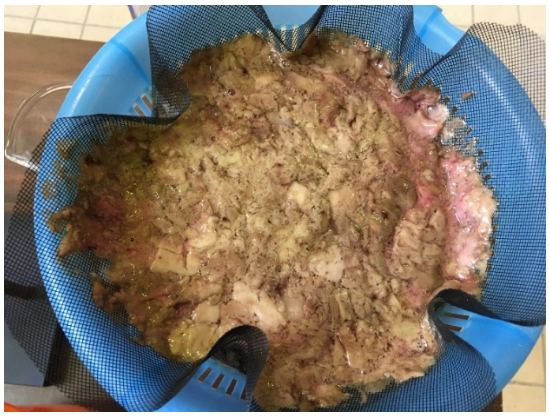

Figure 12: Appearance of chopped and sonicated liver tissue (Exp 2) after 21 h of rendering. without agitation, the oil cannot drain and forms pools throughout the tissue

China cap filter. A china cap filter is similar to a coffee filter but larger and thicker. It was used as a secondary filter to remove fine sludge from the water/oil mixture that drained through the 0.5 mm mesh. The china cap filter was effective in removing fine sludge which improved the separation of oil and water significantly. However, where tissue treatment (sonication, freeze/thaw, and homogenization) caused advanced tissue degradation, increased amounts of fine sludge were observed. This sludge clogged the filter paper and prevented drainage of the oil. The oil stuck in the filter with fine sludge turned brown within 24 h. A side-by-side comparison of oil drained through the china filter and oil stuck in the filter is shown in Figure 13. The china cap filter is a one-time use consumable.

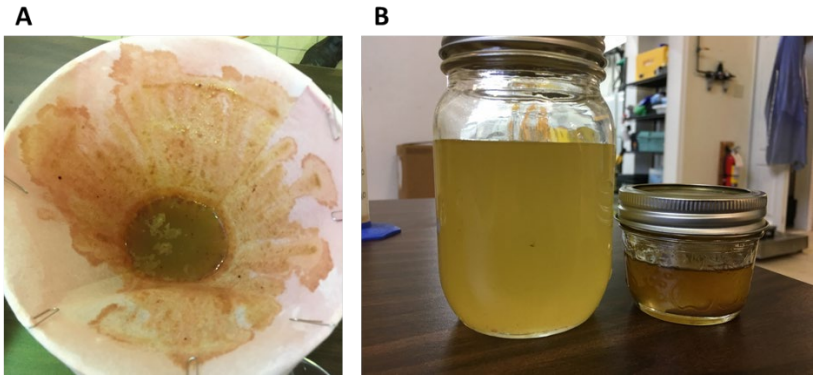

Figure 13: Clogged filter causing visible oil degradation within 24 h under vacuum

Collection flask. In most experiments, the oil/water mixture was collected in a plastic bucket in which the filter or mesh was installed. After the 24 h rendering time, the mixture was carefully poured into a separatory funnel to separate the oil. This caused disruption of the two layers and the mixture was given some time to settle out. A better way to collect the oil without phase disruption would be the design of a decanter-style oil receptacle.

This was attempted in experiment 3. The rendering set-up was to allow oil/water separation during rendering by collecting the liquid in a vacuum flask, allowing oil to overflow into a secondary collection flask (see Figure 2-B). The oil recovered in the secondary flask had a far superior color and clarity than any of the other oils. Unfortunately, due to limitations of the vacuum chamber size, this set-up could not be further optimized.

4.8 Considerations for yield optimization

In this study we have achieved a maximum oil yield of 75%, which can be considered high given the simple experimental set-up and incomplete process optimization.

Yield is at its maximum when the tissue has released the oil but the tissue has not liquefied into fine sludge yet. There is a sweet spot that needs to be established.

- Too much tissue disruption, caused by particle size disruption, sonication, or a combination of the two, results in bad phase separation (loss of oil with sludge phase) and/or clogging of the fine filter.
- Too little tissue disruption delays oil release and extends the exposure of the oil to enzymatic degradation (long rendering time – risk of spoilage during long rendering time)

An optimization of tissue pre-treatment before rendering will likely increase the yield further. In addition, an improved rendering apparatus should be developed to allow optimal draining of released oil from the tissue and through the filter layers.

With such optimizations, we believe it is realistic to achieve an oil recovery exceeding 85%.

4.9 Conceptual design of a pilot-scale rendering system

For the next phase of process development, a pilot-scale batch rendering system should be constructed, tested and modified to allow optimal separation of the oil from the livers. It will allow the production of larger volumes of oil to conduct refining studies and to produce market samples.

Based on the observations made in this study, we have identified that a rendering system must be able to:

1. Produce between 10-20 L of cod liver oil per batch
2. Create & hold a vacuum of 22 in Hg
3. Allow gentle agitation of the liver tissue
4. Allow the continuous separation of oil from tissue and sludge
5. Allow the separation of oil from water at several time points during rendering
6. Prevent contact of oil or liver tissue with metal

Below (Figure 14), we are presenting a conceptual design for such an apparatus.

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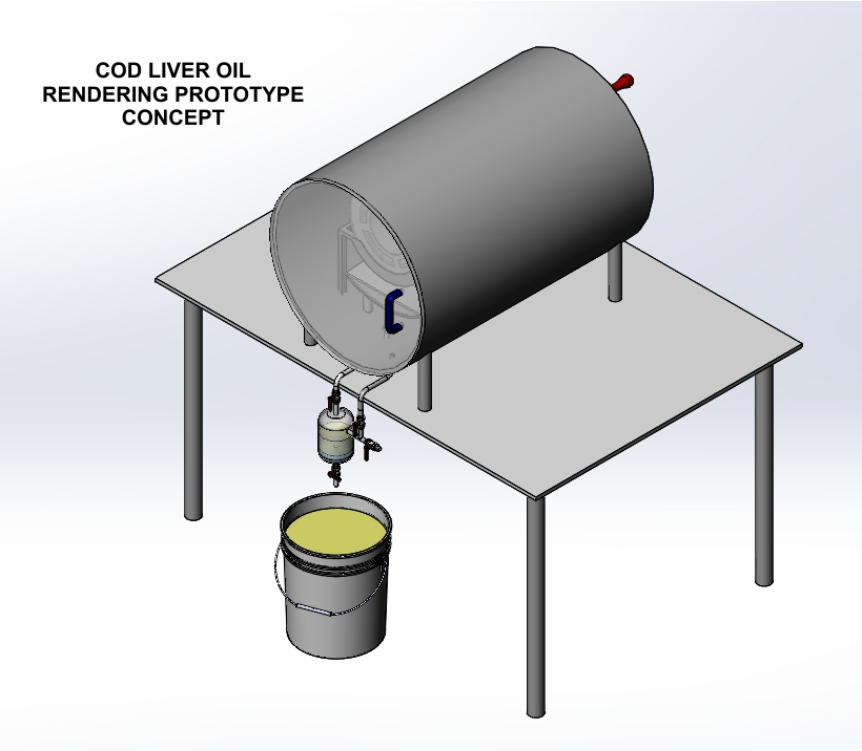

Figure 14: Pilot-scale rendering apparatus - Full assembly

As shown in Figure 14, the apparatus consists of a vacuum chamber into which the filtration system is inserted. The chamber is designed to hold a rough vacuum at -22 in Hg. The filtration system itself is made entirely out of plastic and consists of two horizontal, cylindrical filters that fit into each other (Figure 15).

The inner mesh drum holds the livers. Any released oil and water will drip through the 0.5 mm mesh onto the filter paper membrane which is supported by the paper filter cage. The majority of the liver tissue is retained by the inner mesh, however, some fine sludge will pass through. The paper filter will then remove fine sludge while the liquid drips into the catch basin that sits below the filter drum. The catch basin is raised at the back so the liquid can drain through the pipe into the collection vessel (Figure 16).

The operator can monitor the rendering progress through the transparent chamber door and can occasionally drain the liquid without disturbing the water/oil interface (Figure 14 and Figure 16). To drain, the operator separates the collection vessel from the vacuum of the main chamber by closing the top valve. The vacuum in the collection vessel is then broken by opening the side valve and the liquid can be drained. To avoid oxygen entering the system, the vacuum can be broken using Nitrogen. After draining of the liquid, vacuum in the collection vessel is reapplied (Figure 16) and the top valve opened.

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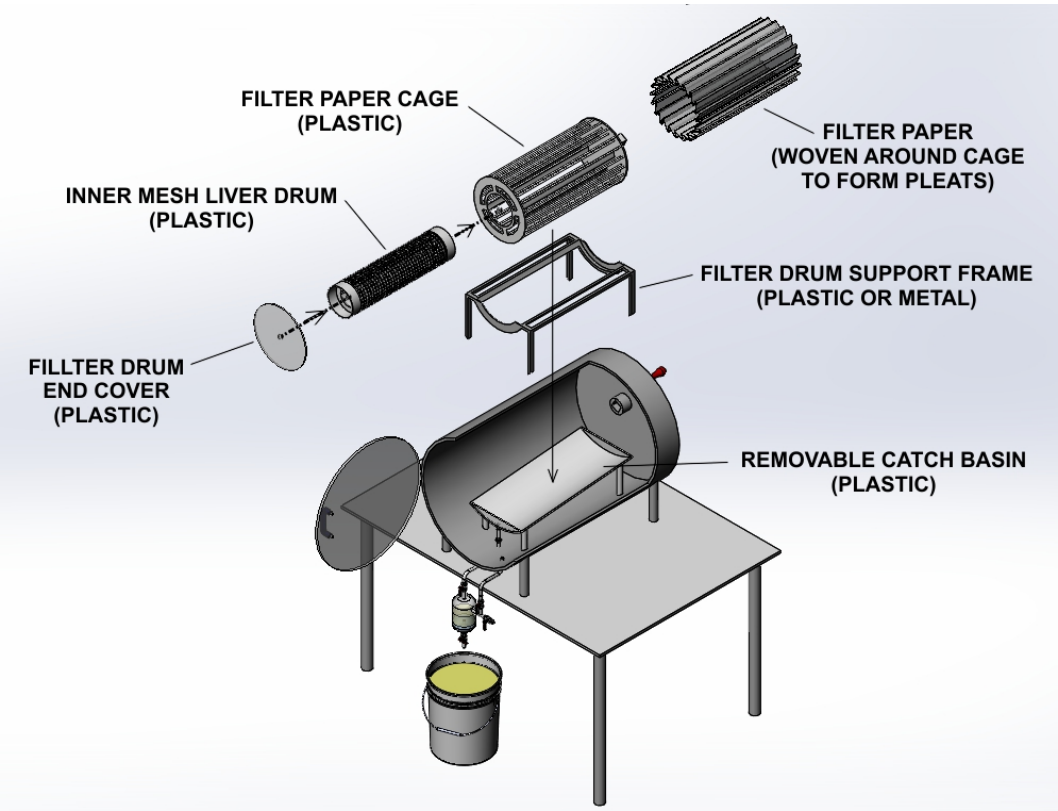

Figure 15: Rendering assembly - exploded

A manual crank allows the operator to independently rotate the inner mesh and the filter paper cage (Figure 17). This serves two purposes: (1) to gently agitate the liver tissue to prevent the formation of pools of oil and (2) to prevent the clogging of the filter paper with fine sludge.

A prototype design should always allow for modifications to be made as the system undergoes testing. Therefore, the vacuum chamber is just a hollow shell and independent from the internal set-up. It's exact shape and dimensions can be adjusted after the internal assembly has been optimized.

All components of the assembly that come into contact with the liver tissue or the oil will be made of plastic. This includes the inner mesh, filter paper cage as well as the catch basin, piping and the oil collection vessel. The internal system consists of separate parts that can all be removed for cleaning.

The frame to support the filter drum straddles the catch basin. This frame could be plastic or metal since the oil never touches it.

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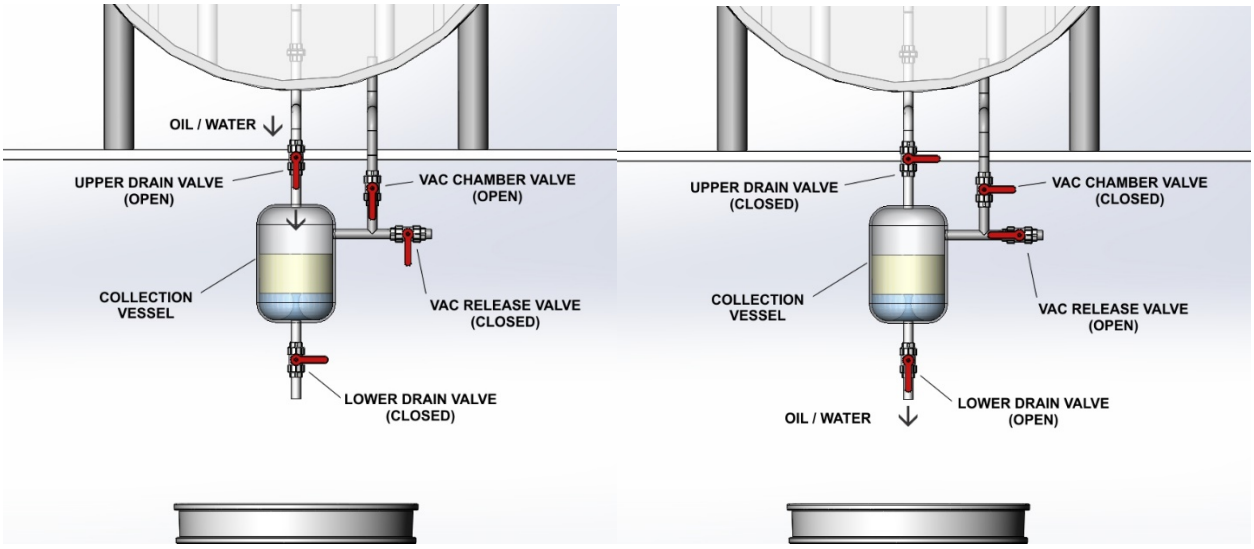

Figure 16: Draining assembly – showing valve settings for accumulating (left) and draining oil (right)

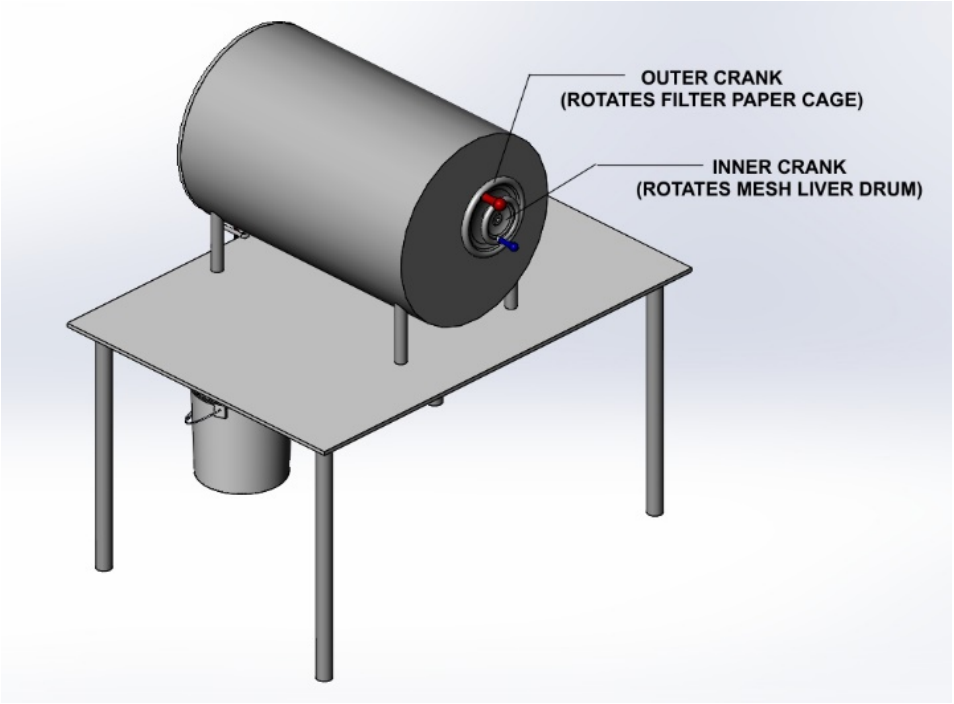

Figure 17: Rendering assembly rear – cranks for rotating the inner assembly

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4.10 Oil Analysis

Quality parameters for fish oils are defined by the WHO standard for fish oils CXS_329e which has been adopted in its current form in 2017. Maximum allowable contaminant levels (heavy metals) are defined by CODEX Stan 193-1995. In addition, the GOED voluntary monograph has been developed as a common guideline for high quality fish oils. In this study we are comparing the cold extracted cod liver oil to these standard specifications.

We selected the two "best" experiments based on oil appearance (smell, clarity, colour) and oil yield (Exp 2 and 9) and analyzed these two samples in depth. Sample #11 was included in the in-house oxidation/FFA analysis in order to see if the quality difference noticed during raw material inspection could be confirmed in the laboratory. The results are shown in Table 4.

4.10.1 Appearance

Our two "best" samples (#2 and #3) showed optimal appearance parameters. The oil was clear and light yellow in colour. The smell was bland and not fishy. Sample #11, and the Rosita market sample had a slightly fishy smell.

4.10.2 Hydrolytic damage (FFA and Acid value)

Hydrolytic damage is assessed by quantifying the % of Free Fatty Acid and then multiplying the result with 1.99 to obtain the Acid value (AV). Hydrolysis occurs when the glycerol backbone of the triacylglycerol is separated from the fatty acid chains, producing Free Fatty Acids. There are three main factors that cause hydrolytic damage: enzymatic oil degradation, long storage, high heat.

The Acid value was above the GOED specifications (5 mg KOH/g) for all four samples analyzed with the lowest values obtained for #2 and #3 (6.44 and 5.37 mg KOH/g, respectively), and the highest value for the Rosita market sample (13.09 mg KOH/g). Sample #11 had an acid value of 9.83 mg KOH/g.

It can be assumed that the hydrolytic damage detected in our process samples (#2, #3, #11) was primarily due to contact of the oil with active liver enzymes during rendering. In the case of the Rosita sample, storage time and/or improper storage conditions may also be contributing factors.

Phase II study should evaluate the effect that improved drainage of oil during rendering has on the acid value. Furthermore, the use of stabilizers will be evaluated.

The acid value being above specifications is of no great concern for a couple of reasons: The specifications are very tight and difficult to meet in a research facility where the production process is not fine-tuned. Furthermore, no oil stabilizer was used at this time. However, in our study, we did show the high importance of using only the best raw material (Higher Acid value in Exp 11) and improving oil drainage during rendering. We expect that the acid value can be kept below 5 mg KOH/g once the entire process including liver collection logistics, is fully designed & fine-tuned.

Another possible reason for elevated FFA is a higher phospholipid content. As no degumming was performed, all natural phospholipids are still contained within the oil. Higher levels naturally drive up the Acid value, which is recognized by the GOED. For example, for fish oils with a phospholipid content >30% (krill oil), the acid value limit is set at 45 mg KOH/g. Phase II should investigate the phospholipid content in more detail.

4.10.3 Oxidation

Oxidative damage is assessed by determining the peroxide value (primary oxidation products) and the p-anisidine value (measuring secondary oxidation products - Ketones). The two values are interrelated. In the early stages of oxidation, peroxides are produced, while ketones are only present at very low levels. As degradation proceeds, peroxides are metabolized into ketones; as a consequence, the peroxide value decreases and the anisidine value increases. The TOTOX value is calculated from both those values and describes the overall oxidation status of an edible oil.

The test results show very low levels of oxidative damage to the oil in samples #2 and #3, confirming the excellent quality of the raw material and the positive effect of the oxygen-free rendering set-up. The TOTOX value of samples #2 and #3 were 8.47 and 8.06, respectively, which was significantly lower than values for sample #11 and the Rosita market sample (22.57 and 24.54, respectively). The elevated TOTOX value in sample #11 was primarily driven by the peroxide level, indicating early stages of oxidation. In contrast, the high TOTOX value in the Rosita market sample was driven by the Anisidine value, indicating a later stage of oxidation where peroxides have been metabolized into ketones. This can be explained by the longer storage time of the Rosita sample in comparison to the experimental samples.

The superiority of the TOTOX value of samples #2 and #3 over of the Rosita sample should therefore not be overrated as the Rosita oil was purchased in store and was therefore not as fresh as our process samples. Some degradation will always occur over time.

The oxidation results emphasize the importance of liver handling, timely processing, storage, and stabilization. Sample #11, which appeared of slightly worse quality than samples #2 or #3 during raw material inspection, showed markedly higher peroxide levels that were outside the GOED specifications.

4.10.4 Vitamin content

Samples #2 and #3 were analyzed for their Vitamin D and A content and both samples fulfilled specifications. In particular, Vitamin A content was twice the minimum required amount. Compared to the levels claimed by Rosita, our samples have a higher vitamin A content and a slightly lower vitamin D content. However, we did not test the market sample for vitamins due to budget constraints.

4.10.5 Fatty Acid Profile

The composition of fish oil depends on what the fish eats. A natural diet full of marine oils will produce a fatty acid profile that fits the fatty acid profile provided in the GOED monograph. If a fish eats a vegetable-based diet, as sometimes occurs in aquaculture, the fatty acid profile will change and look more like a

vegetable oil. As was expected for wild cod liver oil, both samples in our study showed a natural fatty acid profile, with very small exceptions. In sample #2, eicosenoic acid and cetoleic acid were slightly lower than specifications. In sample #3, only cetoleic acid was slightly too low. Eicosenoic acid was also too low in the Rosita market sample. Cetoleic acid has a role as an omega-3 catalyst, stimulating the conversion of short chain omega-3 fatty acids to the healthy, long chain polyunsaturated acids.

Both study samples (#2 and #3) had higher EPA, DHA and DPA levels than the Rosita market sample.

4.10.6 Environmental contaminants

Levels for cadmium, lead and mercury were undetectable in all three samples tested. However, all three samples tested in our study (#2, #3, #11) were significantly above the 0.1 ppm limit (GOED) for Arsenic (2.56, 2.26, 1.99 ppm, respectively). Because the total elemental Arsenic was quantified, it is unknown, how much of the arsenic was present as low toxicity organic arsenolipids and how much as toxic inorganic arsenic.

Arsenic uptake in humans is mainly through foodstuff. Among foodstuff, seafood and fish oils contain the highest levels. Marine fish are more contaminated than fresh water fish (Mania *et al.*, 2015) and higher accumulations are found in species higher up the food chain. Arsenic is present in a multitude of different organic and inorganic forms, which have varying levels of toxicity in humans. Arsenic in fish oils is present mainly as a mixture of Arsenolipids of varying fatty acid length, which are generally regarded as having low toxicity (Mania *et al.*, 2015). Generally, inorganic Arsenic (iAs) is more toxic than organic forms. iAs is generally found at very low levels in fish oils and seafood products (except some seaweed and algae).

While it was long assumed that arsenolipids are of low toxicity, it is now known that they are metabolized into Dimethylarsinite (DMA) which his highly toxic and considered to be central to toxic action of Arsenic (Molin *et al.*, 2015). The majority of the Arsenic metabolites are excreted in the urine within hours of consumption, but is unclear how much damage it causes while in the body. Research into the precise metabolic pathways and the toxic effects of these substances is still in its infancy (Rumpler *et al.*, 2008; Molin *et al.*, 2015; Pereira *et al.*, 2016).

A study by Mania *et al* (2015) tested fish samples available on polish markets and found an average Arsenic concentration of 1.48 ppm, which was comparable to other European countries at the time (Mania *et al.*, 2015).

The phase II study should address the question of how much inorganic and organic arsenic is present in cod liver oil. Arsenic levels can be successfully reduced during oil refining by the same methods suitable for the removal of PCB's, dioxins and Furans. As discussed below, such a refining step will be investigated in phase II.

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Table 4: Analytical results of "Extra Virgin Cod Liver Oil" samples

Quality parameters	WHO 2017 Codex Standard for Fish Oils CXS_329e	GOED Voluntary Monograph for fish oils	Cold-extracted cod liver oil			Rosita™ market sample
			Exp 2	Exp 9	Exp 11	
Free Fatty Acids (FFA)	-		3.24 ± 0.06	2.70 ± 0.04	4.94 ± 0.07	6.58 ± 0.04
Acid value (AV) (1.99 x FFA%)	≤ 3 mg KOH/g		6.44	5.37	9.83	13.09
Peroxide value (PV)	≤ 5 meq/kg	5 meq/kg	3.57 ± 0.04	3.10 ± 0.15	9.51 ± 0.72	1.95 ± 0.26
Anisidine value (p-AV)	≤ 20	20	1.33 ± 0.01	1.86 ± 0.12	3.55 ± 0.08	20.64 ± 0.20
ToTox	≤ 26	26	8.47	8.06	22.57	24.54
Phospholipids	-					
Water content*	-		<50 ppm	< 50 ppm	n.d.	n.d.
Color		colorless, pale, light-yellow to orange	light-yellow	light-yellow	light-yellow	light-yellow
Smell		bland to mild fish-like	bland	bland	mildly fishy	mildly fishy
Nutritional Parameters						
Vitamin A**	≥ 40 µg/ml (retinol equivalents)		77.96 µg/ml	98.99 µg/ml	n.d.	claim: 15.21-25.36 µg/ml
Vitamin D (1 µg = 40 IU)**	≥ 1.0 µg/ml		1.17 µg/ml	1.97 µg/ml	n.d.	claim: 2.03-2.54
Fatty acid composition (%)						
C14:0 myristic acid	2.0 - 6.0		3.41 ± 0.11	3.41 ± 0.16	n.d.	3.72 ± 0.17
C15:0 pentadecanoic acid	ND - 0.5		0.31 ± 0.00	0.31 ± 0.01	n.d.	0.32 ± 0.01
C16:0 palmitic acid	7.0 - 14.0		11.53 ± 0.15	11.73 ± 0.48	n.d.	9.92 ± 0.29
C16:1 (n-7) palmitoleic acid	4.5 - 11.5		7.64 0.05±	7.74 ± 0.04	n.d.	9.67 ± 0.09
C17:0 heptadecanoic acid	NA		0.20 ± 0.00	0.19 ± 0.01	n.d.	0.07 ± 0.10
C18:0 stearic acid	1.0 - 4.0		2.87 ± 0.04	2.86 ± 0.09	n.d.	1.96 ± 0.04
C18:1 (n-7) vaccenic acid	2.0 - 7.0		5.15 ± 0.02	5.35 ± 0.03	n.d.	4.94 ± 0.06
C18:1 (n-9) oleic acid	12.0 - 21.0		17.23 ± 0.13.	18.47 ± 0.36	n.d.	16.59 ± 0.07
C18:2 (n-6) linoleic acid	0.5 - 3.0		1.11 ±0	1.01 ± 0.01	n.d.	2.23 ± 0.02
C18:3 (n-3) linolenic acid	ND - 2.0		0.51 ± 1	0.43 ± 0.00	n.d.	0.82 ± 0.01
C18:3 (n-6) γ-linolenic acid	NA		0.13 ± 0.00	0.13 ± 0.00	n.d.	0.19 ± 0.01
C18:4 (n-3) stearidonic acid	0.5 - 4.5		1.57 ± 0.01	1.33 ± 0.03	n.d.	2.68 ± 0.04
C20:0 arachidic acid	NA		0.21 ± 0.00	0.21 ± 0.02	n.d.	0.13 ± 0.00
C20:1 (n-9) eicosenoic acid	5.0 - 17.0		7.72 ± 0.11	7.40 ± 0.12	n.d.	12.03 ± 0.01
C20:1 (n-11) eicosenoic acid	1.0 - 5.5		0.96 ± 0.10	1.15 ± 0.02	n.d.	0.92 ± 0.11
C20:4 (n-6) arachidonic acid	ND - 1.5		0.55 ± 0.01	0.60 ± 0.01	n.d.	0.42 ± 0.00
C20:4 (n-3) eicosatetraenoic acid	ND - 2.0		0.65 ± 0.01	0.58 ± 0.01	n.d.	0.69 ± 0.00
C20:5 (n-3) eicosapentaenoic acid (EPA)	7.0 - 16.0		11.45 ± 0.02	11.28 ± 0.32	n.d.	8.60 ± 0.19
C21:5 (n-3) heneicosapentaenoic acid	ND - 1.5		0.46 ± 0.01	0.43 ± 0.02	n.d.	0.39 ± 0.01
C22:1 (n-9) erucic acid	ND - 1.5		0.71 ± 0.01	0.70 ± 0.04	n.d.	0.73 ± 0.01
C22:1 (n-11) cetoleic acid	5.0 - 12.0		4.39 ± 0.06	4.26 ± 0.08	n.d.	5.48 ± 0.07
C22:5 (n-3) docosapentaenoic acid (DPA)	0.5 - 3.0		1.32 ± 0.03	1.25 ± 0.04	n.d.	1.03 ± 0.02
C22:6 (n-3) docosahexaenoic acid (DHA)	6.0 - 18.0		12.91 ± 0.50	12.17 ± 0.45	n.d.	10.21 ± 0.41
Total Omega 3			29.02 ± 0.50	27.63 ± 0.88	n.d.	24.63 ± 0.69
Total Omega 6			3.39 ± 0.03	3.27 ± 0.01	n.d.	4.43 ± 0.02

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Total EPA + DHA (mg/g oil)			124.15	113.62	n.d.	106.95
Total PUFA			33.81 ± 0.52	32.33 ± 0.85	n.d.	29.97 ± 0.64
DHA/EPA			1.13 ±0.04	1.08 ±0.01	n.d.	1.19 ± 0.02
O3/O6			0.12 ± 0.00	0.12 ± 0.00	n.d.	0.18 ± 0.01
Contaminants	**CODEX STAN 193-1995 as required by WHO standard CXS_329e**	**GOED Voluntary Monograph for fish oils**				
Arsenic (inorganic As)	< 0.1 ppm	< 0.1 ppm	2.56^{+}	2.26^{+}	1.99^{+}	n.d.
Cadmium	Not regulated for fish oils	< 0.1 ppm	< 0.01	< 0.01	< 0.01	n.d.
Lead	< 0.1 ppm	< 0.1 ppm	< 0.01	< 0.01	< 0.01	n.d.
Mercury	Not regulated for fish oils	< 0.05 ppm	< 0.005	< 0.005	< 0.005	n.d.
PCB's		0.09 mg/kg	1.25 mg/kg	n.d.	n.d.	n.d.
Dioxins and furans		1.75 pg/g (WHO-TEQ)	0.260 pg/g	n.d.	n.d.	n.d.
dioxin-like PCB's		3 pg/g (WHO-TEQ)	3.47 pg/g (WHO-TEQ)	n.d.	n.d.	n.d.
Total POP's (sum of dioxins, furans and dioxin-like PCB's)		3 pg/g (WHO-TEQ)	3.73 pg/g (WHO-TEQ)	n.d.	n.d.	n.d.

*n.d.: not determined; * The water content was determined using the Sandy Brae water test kit™ and water levels were below detection limit of 50 ppm for both samples. **unit conversion based on cod liver oil density of 0.93 g/ml. $^{+}$ Total Arsenic (organic and inorganic)*

4.10.7 Dioxins, Furans, and PCB's

The contamination of marine oils with persistent organic pollutants or POP's (PCB's and dioxins/furans) has been a concern for many years. Because these POP's accumulate in an organism over time, species higher up in the food chain are most affected. Furthermore, because POP's are fat soluble, they accumulate in fatty fish species or species that store their fat in the liver like cod.

Most commercially sold fish oil is subjected to a refining step that removes those POP's. If POP concentration is low, additional refining is not needed. Unfortunately, at CA $2200 per sample for dioxin, furan and PCB analysis, regular monitoring of POP level is difficult and in this study we could only test one sample.

However, the results clearly showed that further refining of the oil is needed. At 1.26 mg/kg, the total PCB concentration was 14 x the maximum allowable concentration of 0.09 mg/kg. The concentration of dioxin-like PCB's was 3.47 pg/g WHO-TEQ, also clearly exceeding the allowable limit of 3 pg/g WHO-TEQ.

A study by John (2013) tested 1894 fish oil samples from all over the World for heavy metals including Arsenic, PCB's, dioxins & furans, as well as dioxin-like PCB's. They found the following mean values for persistent environmental pollutants (John *et al.*, 2013):

- Total As: 0.12 ppm
- PCB's: 24.54 ppb
- Dioxins & furans: 0.55 ppt WHO-TEQ

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- Dioxin-like PCB's: 0.84 ppt WHO-TEQ

They noted that less concentrated oils (<50% EPA + DHA) were more polluted (mercury and PCB's) but within GOED tolerance.

Jacobs (1998) found cod liver oil + concentrated fish oil samples to have the highest PCB levels, however, all samples were below the US FDA limit for total PCB's of 2.0 ppm at the time (todays limit: 0.09 ppm). (Jacobs *et al.*, 1998). Fernandez (2013) identified that cod liver oil had the highest levels of POP's, characteristic for oil extracted from the liver (Fernandes *et al.*, 2006).

4.11 Considerations for refining

Based on the quality analysis of the extracted oil, refining of the cold-extracted cod liver oil can be kept to a minimum due to the excellent freshness of the raw material, proper handling, storage and timely processing. Therefore, no refining steps are needed that address parameters related to oil degradation (hydrolytic or oxidative damage, smell or color deterioration).

A winterization step is recommended to improve product appearance (clarity, reduction of sediment) and to increase the omega-3 content by removing non-omega-3 components of the oil.

Due to the significant levels of POP's, we also recommend a refining step (e.g. activated carbon filtration) that is suitable to reduce PCB and arsenic levels by at least 90%.

In the interest to refine the oil at low temperatures to preserve its nutritional quality and as discussed in the introduction, we will be looking at membrane technology to achieve the above mentioned reduction in POP's.

5 CONCLUSIONS

In this study, we proved that it is feasible to extract high-quality cod liver oil at low temperatures (<17°C) by using a modified, natural rendering process and by following a highly detailed raw material procurement procedure.

The following factors were determined to be most critical for maximizing oil quality:

- Raw material freshness
- Handling, cleaning, and storage of livers
- A fine-tuned process to weaken fatty tissue, consisting of a combination of cryo- and ultrasonic-treatments
- Rendering under vacuum
- The separation of oil from water and tissue sludge during rendering

The resulting oil was of exceptional sensory quality and featured a light yellow colour, clear transparent appearance, and a neutral non-fishy smell.

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An oil yield of 75% was achieved in this preliminary study. It can be expected that the yield can be further increased to >80% by fine-tuning the rendering apparatus. A conceptual design for a pilot-scale rendering apparatus was developed and is included in this report.

Significant levels of POP's and total arsenic were measured which prescribe the requirement for a refining step that is able to reduce these contaminants by at least 90%.

6 RECOMMENDATIONS

We make the following recommendations based on this analysis.

1. To design a Phase II project which will address:
    - 1.1 The design and construction of a pilot-scale rendering system;
    - 1.2 The fine-tuning of the developed process on a pilot-scale;
        - 1.2.1 Optimization of oil drainage and filtration during rendering
        - 1.2.2 Optimization of cryo- and ultrasonic pre-treatment
    - 1.3 The development of a refining step to remove POP's;
        - 1.3.1 Preferably using membrane technology at moderate temperatures.
    - 1.4 The production of test market samples for the industry client.

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