Concentrating omega-3 oils – Supercritical fluid technology versus molecular distillation

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INTRODUCTION

During the last three to five years there has been a clear shift from standard "18/12" fish oils (where "18" stands for "**18% EPA**" and "12" stands for "**12% DHA**") to concentrated "omega-3 oils". At first the concentration of EPA and DHA went up from approximately 30% to 55%. Then individual omega-3 fatty acids like EPA or DHA were offered at 70%, then at 90% and today there are a handful of companies that are able to produce EPA and DHA on an industrial scale to concentrations of more than 95%. Select companies like KD-Pharma GmbH / Bexbach in Germany can concentrate omega-3 oils even further, producing EPA oils up to 99%.
This paper will discuss the general path of concentrating omega-3 fatty acids with special emphasis on the two most important techniques: **Supercritical Fluid Extraction** (SFE) combined with **Supercritical Fluid Chromatography** (SFC) and **Molecular Distillation** (MD).

ORIGIN OF THE FISH OIL

All fish oil processing starts with **fishing**. Here the first question that comes up is: where are the fish caught? In the Atlantic, Pacific, or Mediterranean Sea? Among insiders in the omega-3 field, this question is easy to answer. Today the most preferred place to catch omega-3 rich fish is in the South Pacific, close to the shore of Peru and Chile. In this area the cold, unpolluted waters come up from the Antarctic and ensure low levels of contaminants in the fish oil. This does not mean that oils from other areas of the world cannot be adequately decontaminated, but it is preferable to handle fish oil that starts with low levels of contamination. Today standards and requirements for purity are so high that even "clean" oils from the South Pacific undergo a decontamination process before they are sold.
The next question is: what fish are we looking for? Generally we are looking for "blue fish", or in other words, fatty or oily fish. "Blue fish" include sardines, anchovies, tuna, mackerel, etc. The very tasty "white fish" are unfortunately a very poor source of omega-3.
The next question is: are we looking for large or small blue fish. The answer is easy: the shorter the life cycle of the fish, the less time it has to accumulate heavy metals, pesticides, PCB's, dioxins and other environmental pollutants. Larger fish, given their longer life span, typically accumulate higher levels of environmental contaminants which then end up in the oil. Additionally, larger fish are much more valuable as food, therefore, only the inedible portions (i.e. eyes and intestines) of large fish such as salmon and tuna are available for oil production. There is nothing wrong in making use of these "**left overs**" in the production of omega-3 oils, however, the perception that this oil is made from the entire salmon or tuna is misleading and should be revised.
Generally, fish oil producers are looking for small, short life-cycle fish like sardines, anchovies, and menhaden. Due to the short life cycle of these fish and their rapid reproduction capability, a sustainable annual catch can be managed. Furthermore, these small fish offer both fish meal and fish oil. What many people don't realize is that these fish are primarily harvested for fish meal production and the oil is simply a byproduct. Today 90% of all fish oil is a byproduct of fish meal production.
Next question is: where do we catch these small blue fish? It is known that the same species of fish contain more polyunsaturated fatty acids (i.e. EPA and DHA) if they live in cold water. An arctic sardine will contain significantly more EPA and DHA than a Mediterranean sardine. Finally we have to determine which cold water currants contain the lowest level of contaminants. Putting all these criteria together shows us that the vast majority of fish oils and omega-3 concentrates originate from cold water sardines, anchovies or menhaden caught off the shores of Peru, Chile and in case of the Menhaden, in the Golf of Mexico and the Atlantic coast (USA).

FISH OIL PROCESSING

Once caught the small blue fish go through the following standard process steps. Still on board the shipping vessel, they are cooked and pressed. Here the water and oil is separated from the

protein while the solids (proteins) are transformed into fish meal.
Next, the water is removed from the oil and the oil is then deacidified, degummed and washed several times with water ("polishing"). Most of this oil is then used as animal feed. A majority of the feed is applied in aquaculture to ensure that the farmed fish also contain a minimum amount of omega-3 fatty acids. The smaller portion of oil which goes to human consumption undergoes a bleaching and deodorizing process at 160-200°C. The resulting fish oil is known as the classical "18/12 TG", where "TG" stands for **triglyceride**.

DECONTAMINATION

At this stage, the fish oil supplement producer has two options: i) encapsulate the oil as is and sell it to the consumer as a low priced supplement, or ii) decontaminate the oil before encapsulating and selling it to the consumer. The additional decontamination steps create extra costs for the producer, but the end result is a higher quality oil. The decontamination can be done using certain absorbents like activated carbon or silica materials. Other very popular and very effective approaches to decontaminating fish oil include molecular distillation or supercritical fluid technology. At this stage of the process the supplement producer encounters another important question: do I want to sell fish oil or an omega-3 concentrate? After all, the fish oil only contains 30% of the beneficial omega-3 fatty acids while the other 70% of the oil consists of undesired components such as saturated fats, cholesterol, and omega-6 fatty acids. Given the low concentration of the omega-3 fatty acids in the standard fish oil, it makes senses to enrich or concentrate the omega-3 fatty acids.

CONCENTRATION PROCESS

In natural fish oil you will almost never find more than one long chain omega-3 fatty acid connected to one glycerol molecule. The other two positions are normally occupied with shorter and/or more saturated fatty acids. The reason for this structure is that the very large and spacious omega-3 fatty acids EPA and DHA would simply not fit next to each other on one glycerol molecule. Therefore most natural fish oils contain a maximum of about 30% EPA and DHA. If we want to offer a higher omega-3 concentration, we are forced to remove these fatty acids from the glycerol backbone by converting them into free fatty acids, or preferably, into ethyl esters. This is normally done by letting the oil react with a catalyst under the presence of ethanol at temperatures between 80°C and 90°C. Once the fatty acids are liberated from the glycerol backbone there are numerous possibilities to enrich the omega-3 fatty acid concentrations including:

- urea precipitation;
- crystallization at low temperatures;
- **supercritical fluid extraction** (SFE);
- **supercritical fluid chromatography** (SFC);
- high performance liquid chromatography (HPLC/LC); and
- **molecular distillation** (MD).

In the following, the two most important enrichment technologies, supercritical fluid chromatography and molecular distillation will be discussed.

Molecular Distillation

Modern (MD) is very gentle and efficient way to produce moderately concentrated EPA and DHA products. This technology makes use of the fact that the free fatty acids and fatty acid ethyl esters have relatively low evaporation temperatures when placed under a strong vacuum. By heating the oil under vacuum to temperatures of 140°C and 160°C, a good separation between smaller C-18 fatty acids and larger C-18 fatty acids can be achieved. This separation concentrates the initial EPA and DHA content from 30% to approximately 55% and produces an oil which is readily available on the market today.
Further concentration using MD has its limitations. First, increasing the EPA and/or DHA concentration using MD can result in a substantial drop in yield. Producing EPA or DHA concentrates with more than 70% of each individual fatty acid is typically not possible with MD given its lack of selectivity. Another drawback of using MD to produce highly concentrated oils is the necessity to repeatedly run the oil through the MD process thereby exposing the oil to several rounds of heating. Even though literature shows no direct proof that repeated exposure of the oil to high temperatures damages or degrades the EPA and DHA, it is likely that these sensitive products when exposed to repeated thermal stress, will encounter shortened stability and shelf-life when compared to products produced at lower temperatures. Finally, as separation with MD is based on the chain length of a given molecule every "EPA-fraction" will also contain C20:4n3, C20:4n6, C20:3n6 and probably C21:5n3 while all DHA concentrates will also carry their share of C22:5n3, C22:5n6, C22:4n6 and probably C21:5n3.
The use of MD is not limited to the concentration of the free fatty acids or their esters. As mentioned earlier in this paper, MD is an excellent tool to purify fish oil (TG's) from cholesterol and traces of heavy metals, pesticides, PCB's, and dioxins. Certain optimization procedures such as the patented carrier systems/working fluids developed by Pronova have further improved the ability of the MD process to eliminate contaminants (1). Table I shows the advantages and disadvantages of MD compared to Supercritical Fluid Chromatography (SFC).

Supercritical Fluid Technology

Supercritical Fluid Technology (SFT) is characterized by the fact that excludes the use of toxic organic solvents and instead uses compressed carbon dioxide (CO_2) at very low temperatures (40-50°C) to extract and/or to separate the individual fatty acids of a fish oil.
SFT typically employs two key processes in order to refine and concentrate fish oil which are commonly referred to as Supercritical Fluid Extraction (SFE) and Supercritical Fluid Chromatography (SFC).

Supercritical Fluid Extraction

SFE, or more precisely "counter-current SFE" (ccSFE) is a very elegant and continuous technology to refine and concentrate fish oils. On the one hand it can be used to eliminate cholesterol and contaminants like heavy metals, PCB's, dioxins, and other impurities from the

triglycerides and on the other hand it can be used to produce very interesting ethyl ester concentrates as Brunner and co-workers demonstrated in 1996 (2). However, due to economic reasons, SFE is limited to the production of semi-concentrates, having combined EPA and DHA concentrations of about 50-65%.

Supercritical Fluid Chromatography

The industrial SFC technology for the enrichment of omega-3 fatty acids was developed by Lembke in 1993 and later patented and trademarked by KD-Pharma as the "kd-pur" Technology (3). The SFC technology is a highly selective and gentle process working at temperatures in the range of 40°C – 50°C. The low temperature range prevents thermal stress on the highly temperature sensitive EPA and DHA, and renders SFC one of the most suitable technologies for the concentration of poly unsaturated fatty acids. Furthermore, supercritical CO_2 has a very low viscosity which enables the use of long chromatographic columns packed with highly selective packing material. This high selectivity together with the high diffusion coefficient observed in supercritical CO_2, explains the excellent performance of this technology for concentrating omega-3 fatty acids. Additionally, this SFC technology serves very well to eliminate, or further reduce traces of remaining contaminants in the oil.
In contrast to the above discussed SFE process, the high selectivity of SFC enables the industrial production of up to 99% pure individual fatty acids such as EPA-99%. Lately there is some confusion on the market regarding the various supercritical fluid technologies. SFT is the combination of both SFE and SFC. In some instances products on the market are referred to as "Supercritical", however they have been produced using only the SFE purification process. In fact, due to patent reasons, many of these products were produced by the extraction process (SFE) alone and do not achieve the same quality of omega-3 concentrates produced using the two part SFT process.

	Molecular Distillation (MD)	Supercritical Fluid Chromatography (SFC)
Operation temperature (°C)	140 – 160	35 – 50
Operating pressure	0.001 mbar	> 140 bar
Solvent/mobile phase	N.A.	CO_2
Selectivity	Medium	Very high
Max viable concentration achievable (without any other technology applied)	65-75%	99%
Decontamination efficacy	Very high	Very high
Mode of operation	Continuous	Semi-continuous
Risk of product oxidation	Low	Very low
IP Status	Certain operating modes patented	Patented
Flexibility to adjust EPA/DHA composition of final product	Limited	Very high

Table I

CONCLUSION

This paper covers the fish oil concentration process starting from the fishing vessel up to the final concentration technologies used to produce omega-3 concentrates. Most EPA and DHA concentrates on the market were produced by **Molecular Distillation** or **Supercritical Fluid Chromatography** (SFC) – not to be confused by the terms "**Supercritical Technology** (SFT)" or "**Supercritical Fluid Extraction** (SFE)" which lack sufficient selectivity to reach these high concentration levels.
Molecular Distillation (MD) is an excellent technology to decontaminate and remove cholesterol from fish oils triglycerides. However SFE and SFC (especially when combined) are also capable of doing exactly the same. So in this case there is no substantial difference between the two technologies apart from the fact that the investment for the MD is substantially lower than the investment for an industrial SFE and SFC plant. Both technologies MD and SFC claim to be proprietary and covered by patents. The only difference between these two technologies is that fish oil passing through the MD process suffers up to 350% higher thermal stress compared to a product passing through the SFE/SFC technology.
With respect to the concentration process, the SFC has a much higher selectivity and a lower thermal stress on the concentrates when compared to the MD process. When using MD to produce omega-3 concentrates, this requires repeated runs through the MD process, exposing the thermal sensitive EPA and DHA to high temperatures on a repeated basis. So far no substantial essential fatty acid decomposition has been reported in the literature as a result of the conditions employed in MD, however, an increased thermal stress for these highly unsaturated fatty acids may have an impact on their stability and shelf-life.
Both technologies MD and SFC seem to be very well suited for the decontamination and production of omega-3 concentrates. MD seems to have economical advantages in the lower to middle range concentrates going up to an EPA+DHA concentration of about 70%. However for higher concentrations of the individual fatty acids (e.g. EPA 80%, EPA 95%, DHA 95% or more) MD seems to have reached its limits. For these unique high quality products the selective and very gentle SFC technology is the method of choice.

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