19 Production Techniques for Omega-3 Concentrates

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Key Points

- There is a clear trend observable in the omega-3 business: the shift from semi-concentrated omega-3 supplements to highly refined pharmaceutical ingredients.
- The origin of the fish oil has significant impact on the final contamination levels of omega-3 concentrates.
- Molecular distillation (MD) technologies can produce high-quality semi-concentrated omega-3 products; for very high concentrated omega-3 formulations, their limited selectivity is not sufficient.
- Supercritical fluid chromatography (SFC) is especially useful for the production of highly concentrated EPA or DHA products, mainly because of its very mild separation conditions and high selectivity.

Key words: Omega-3 concentrates, Crystallization, Urea precipitation (UP), Molecular distillation, Supercritical fluid extraction and chromatography (SFE/SFC), Liquid chromatography

INTRODUCTION

During the last 20 years there has been a clear shift from standard "18/12" fish oils—where "18" stands for 18% eicosapentaenoic acid (EPA) and "12" stands for 12% docosahexaenoic acid (DHA)—to concentrated "omega-3 oils." It was a gradual process over most of the years, but lately the demand for high concentrated omega-3 oils has increased dramatically. A first step was achieved by eliminating most saturated and

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the demand for EPA+DHA concentrates reaching 85% increased significantly and lately a clear trend to highly concentrated EPA (EPA 96%) is observed, most likely based on the soon-to-be-commercialized Amarin drug AMR101. Today there are only a handful of companies worldwide that are able to produce EPA and/or DHA products with more than 95% purity, but this will change in the near future owing to the growing demand for such products.

This chapter will discuss the most common concentration technologies applied to obtain omega-3 concentrates, with special emphasis on the two important techniques: supercritical fluid technology (SFT), which includes both supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC), and molecular distillation (MD).

FISH OIL (CONCENTRATE) PRODUCTION

Most fish oil for human consumption is a by-product that comes from the fish meal (animal feed) industry. Only approximately 5% of world fish oil production is used as food ingredients, food supplements, or medication. The remaining 95% is used mainly for animal feed, especially in the field of fish farming (Fig. 19.1) (Personal communication with GOED, 2010).

The oil often originates from sardines, anchovies, menhaden, and other small oily fish. Directly after being caught, the fish are often already boiled on board the fishing

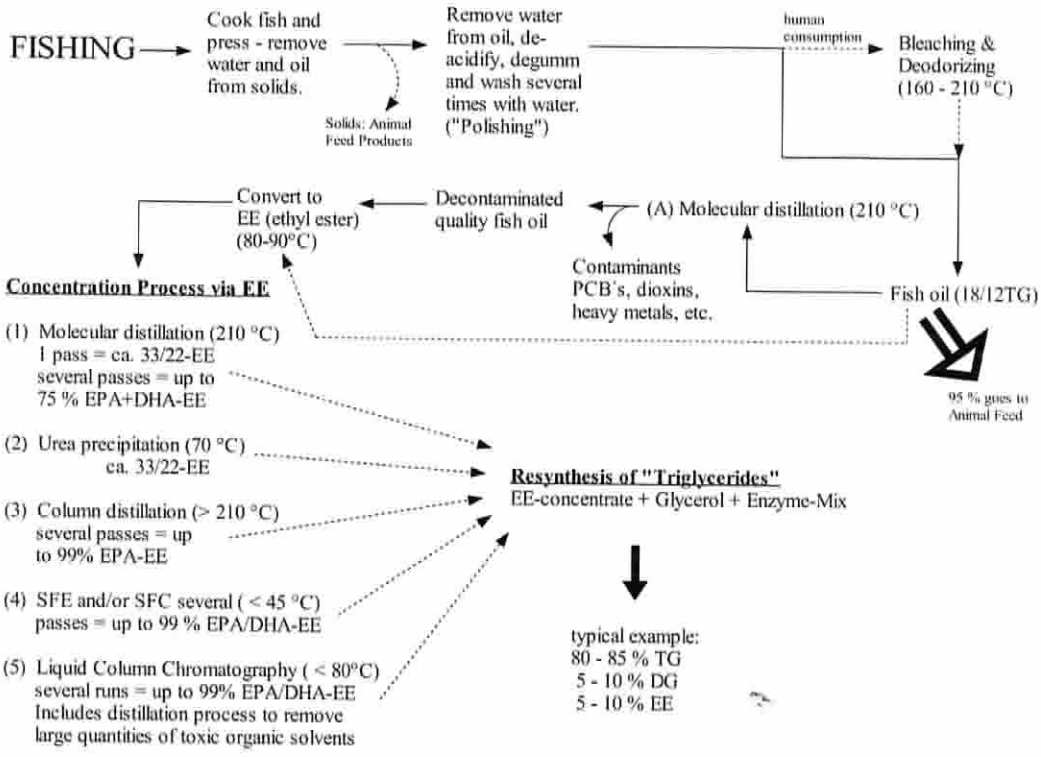

Fig. 19.1. Omega-3 concentrate production pathways.

vessel and then mechanically pressed to remove their body water and fat/oil. The remaining meat and bones are minced, dried, and finally sold as protein-rich animal feed, known as fish meal. The obtained water/oil emulsion is first filtered and then separated into an oil and water phase by large industrial centrifuges. The oil then gets washed several times with hot water, which is known as polishing. Finally, the water is quantitatively removed by centrifugation and the now dried fish oil can be stored.

If the oil is for human consumption, it has to be bleached and deodorized. Bleaching is carried out in the absence of oxygen and is basically the removal of pigments from the oil with the help of adsorbents like Bentonite, activated carbon, and or silica (1). During the subsequent deodorizing step, the undesired fishy smell and taste get removed and the shelf life of the oil is prolonged. Deodorization is often a vacuum distillation process where at about 170–220°C the free fatty acids, oxidization products like ketones and aldehydes, and other lighter boiling substances (compared to triglycerides) are stripped off. Unfortunately, this process does not remove cholesterol, saturated fatty acids, or contaminants like heavy metals, dioxins, pesticides, polychlorinated biphenyls (PCBs), furans, and so forth from the oil.

DECONTAMINATION PROCEDURES

The degree of contamination depends strongly on the location where the fish was caught. Usually the south Pacific (coast of Chile and Peru) and south Atlantic (South Africa) cold water currents, originating from the still largely unpolluted Antarctic, carry much fewer pollutants than, for example, the Gulf of Mexico, North Atlantic, Indian Ocean, or Asian waters. Typical examples are found on the southwest coast of Africa, Chile, and Peru, where the water is known to have very low levels of pollutants. However, if these or other currents pass by heavily industrialized areas, they pick up all kinds of undesired contaminants and pass them on to all living creatures in that area (Fig. 19.2).

Because most of these pollutants are fat-soluble, they accumulate especially in the fatty tissue of the marine animals, including fish. We humans are at the end of the food chain, so the pollutants finally build up in our body fat, brain, and nervous system. Older, larger fish have spent more time in polluted waters, so their body fat is more contaminated. Therefore, from the contaminant point of view, small oily fish with short life-cycles (e.g., sardines, anchovies) are always preferable to large long-living fish like tuna. It is always easier to choose a less contaminated fish oil than to remove these contaminants later from the product. The removal of the contaminants can be done either by chromatographic methods, by selective adsorption techniques (e.g., activated charcoal, aluminum oxide, etc.), or by short path distillation of the fish oil triglycerides (2–4).

CONCENTRATION PROCESS

After removing the contaminants from the fish oil either by absorption process (e.g., with activated charcoal), chromatography, SFE, or short way distillation, the fish oil still contains almost the same EPA and DHA concentration as the raw oil. For example, about 18–20% EPA and 8–12% DHA in the case of sardine oil.

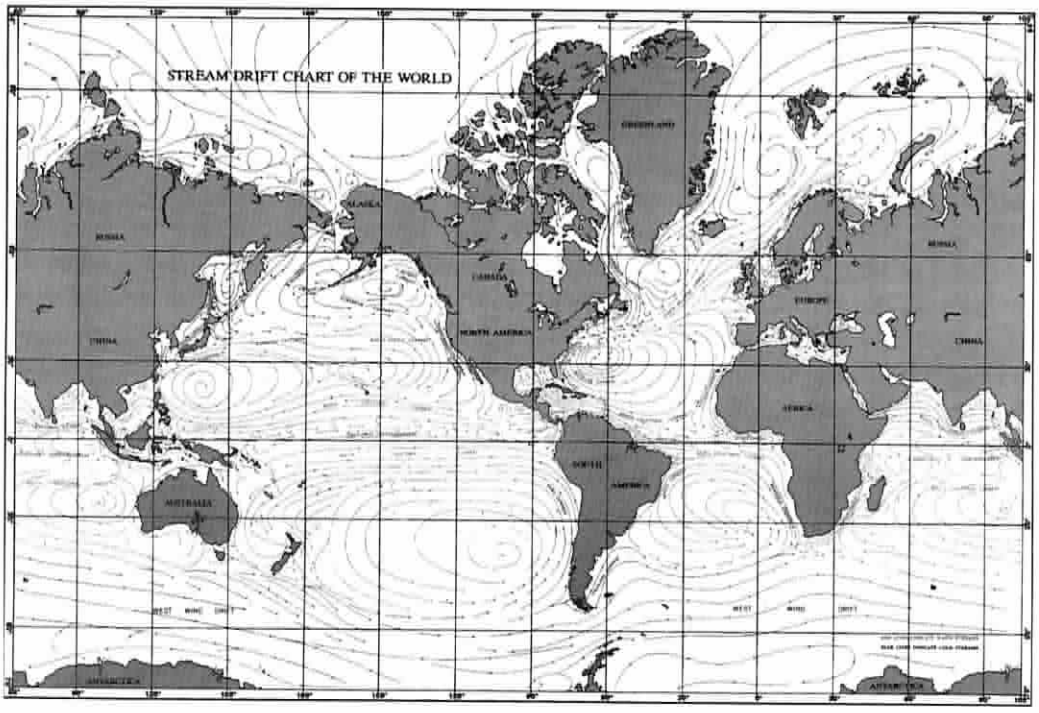

Fig. 19.2. Ocean currents. Reprinted with permission from: http://upload.wikimedia.org/wikipedia/commons/1/16/Ocean_current_2004.jpg.

In order to concentrate any fish oil, the first thing that has to be done is to convert the natural triglycerides into ethyl esters (EE) or free fatty acids (FFA). Only when the EEs or FFAs are removed from the glycerol backbone of the original triglyceride molecule is it possible to enrich their concentrations. This is usually done in a huge reactor by treating the fish oil triglycerides in the presence of an alkaline catalyst with pure ethanol for at least an hour at about 80°C. This process has been applied in the fish oil industry for more than 100 years. This esterification reaction can, of course, also be done with help of specific enzymes (lipases) in the presence of pure alcohol.

Once they have been liberated from the glycerol backbone, there are numerous ways to enrich the free omega-3 fatty acids or ethyl esters, such as the following:

- Enzymatic enrichment
- Urea precipitation
- Crystallization at low temperatures
- Supercritical fluid extraction (SFE)
- Supercritical fluid chromatography (SFC)
- High-performance liquid chromatography (HPLC/LC)
- Molecular distillation (MD)

The most important enrichment technologies for the production of omega-3 concentrates will be discussed in more detail in the following sections.

ENZYMATIC ENRICHMENT

An elegant and very gentle way to enrich fish oils is to liberate the FFA from the glycerol backbone with help of specific enzymes that only cleave in the sn1 and sn3 position of the glycerol or that cleave specifically in the sn2 position (5, 6). As most fish oils have the majority of their long-chain omega-3 fatty acids (EPA and DHA) in position sn2 of the triglycerides and phospholipids, a specific sn1/sn3 enzyme (e.g., lipase B from *Candida antarctica*) added to a fish oil produces two fractions: (1) free fatty acids (from sn1 and sn2) and (2) a 2-monoacylglycerol enriched with EPA and/or DHA (7, 8). The FFA can easily be removed by MD, leaving a concentrated omega-3 rich 2-monoacylglycerol fraction. To this fraction previously enriched EPA and DHA-FFA could be added together with a lipase, which synthesizes an omega-3 rich reconstituted triglyceride product. With this enzymatic enrichment technology, omega-3 concentrations (EPA + DHA) of approximately 50% and higher are achievable.

UREA PRECIPITATION

The unique characteristic of urea crystals to incorporate straight chain molecules, such as saturated fatty acids, was first patented in Germany by Bengen in 1940 (9) and revolutionized the lipid industry. In 1955, Domat et al. described a urea process for the fractionation of marine oil fatty acids using this technology (10). It is a simple process: urea gets dissolved in a hot organic solvent (e.g., methanol, ethanol, and water mixtures thereof), the fatty acid or EE mixture is added, and then the entire solution is cooled down. During the cooling process the urea crystallizes again and forms urea-fatty acid complexes, trapping the straight chain saturated, and partly also mono-unsaturated, fatty acids and, hence, separating them from the curved polyunsaturated fatty acids (every C=C double bond induces a 40-degree angle in the fatty acid chain). After filtration and washing of the urea crystals a concentrate of the highly unsaturated fatty acids is obtained. According to Ratnayake et al. (11) the optimal ratio between menhaden oil free fatty acids and urea is 1:3, and the optimal cooling temperature is 1°C. Urea fractionation was considered for many years as an interesting tool for producing omega-3 concentrates containing approximately 45–60% EPA + DHA. It has a high selectivity towards saturated and mono-unsaturated long-chain fatty acids but often requires the handling of large volumes of inflammable solvents and water. In addition, huge amounts of the urea-saturated FA complex have to be disposed, making this technology relatively expensive. Nowadays other technologies (e.g., molecular distillation) have replaced former urea precipitation plants.

CRYSTALLIZATION

Low-temperature crystallization of lipids, especially triglycerides and fatty acids, in organic solvents is also one of the very old techniques for concentrating fatty acids (12, 13). This technique separates the triglycerides and fatty acids or their methyl/EEs according to their melting points in different organic solvents like hexane, methanol, ethanol, or acetone at very low temperatures: −50 to −70°C. The low-temperature crystallization process has to utilize large amounts of inflammable solvents, which

presents a serious drawback for large-scale industrial applications. Nevertheless, there are companies producing highly concentrated omega-3 oils that have specialized in using this crystallization technology in combination with other purification processes, like HPLC.

SUPERCRITICAL FLUID EXTRACTION (SFE)

SFE or, to be precise, counter current-SFE (cc-SFE) is a very elegant method to

1. Decontaminate fish oil triglycerides
2. Remove polar impurities, color, FFA, oxidation products from the EE fraction, and
3. Enrich the C20 and C22 groups of fatty acids

The so-called supercritical fluid is normally carbon dioxide (CO_2). If CO_2 is compressed above a pressure of 73 bar at a temperature above 32°C it turns into the supercritical state. In this state CO_2 is an excellent solvent for most lipids. Polar compounds are insoluble in CO_2 and can thus be separated. Once depressurized the CO_2 loses its dissolving properties and the pure lipids/FFA/EE drop out of the gaseous CO_2 without any dilution, as found when using liquid organic solvents.

The solubility of fatty acids in supercritical CO_2 decreases with increasing molecular weight. The longer the fatty acid, the less soluble it is in supercritical CO_2. Therefore, cc-SFE can also be used, for example, for a selective separation between all C18, C20, and C22 fatty acids, as Brunner et al. showed in 1996 (14).

However, cc-SFE cannot distinguish very well between the degrees of saturation within a particular group (e.g., C20). Therefore, as with molecular distillation, owing to other fatty acids with different degree of unsaturation within the C20 or C22 group, it will not be possible to achieve very high EPA or DHA purities, while working with acceptable yields, using cc-SFE alone.

In a simplified way, cc-SFE is nothing but a long vertical column; supercritical CO_2 is pumped into the column from the bottom, under high pressure (>73 bar). The CO_2 flows from the bottom to the top, where it leaves the column and eventually is recycled and pumped back into the bottom of the column. With help of a second pump, the feed (fish oil ethyl ester) is continuously pumped into the upper part of the separation column and falls down in counter-current direction of the rising CO_2 due to gravity. The components bottom of the column and are collect there. Consequently, cc-SFE can only separate a mixture into two fractions: a CO_2-soluble fraction and a CO_2-insoluble fraction. However, by changing the operating pressure and temperature within the supercritical region, and thus the density of the CO_2, it is possible to adjust the selectivity of this cc-SFE process in such a way that even two CO_2-soluble components can be successfully separated.

The cc-SFE process has several advantages: it is a very gentle technology, it does not use any toxic organic solvents, it operates at low temperatures (usually below 45°C), there is no dilution observed after separation (which eliminates evaporation costs when

using any kind of liquid organic solvents in extraction or chromatography processes), and the highly sensitive omega-3 fatty acids are always under a protective blanket of CO_2, avoiding all possible contact with oxygen and, thus, oxidation.

The main disadvantages of this technology are the limited selectivity and the high investment costs for the pressure equipment.

SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC)

Supercritical fluid chromatography is basically the same as liquid chromatography (LC), but instead of using toxic organic solvents as "mobile phase" it uses compressed CO_2.(15) The large-scale industrial SFC technology for the enrichment of omega-3 fatty acids was developed by Lembke in 1994 (16) and later patented and trademarked by KD-Pharma as the "kd-pur" Technology. (17–19) The SFC technology is another highly selective and gentle process working at temperatures in the range of 40–50°C. The low temperature range prevents thermal stress on the highly temperature sensitive EPA and DHA, and makes SFC one of the most suitable technologies for the concentration of polyunsaturated 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. (20) Additionally, this SFC technology serves very well to eliminate or further reduce traces of remaining pollutants in the oil.

In contrast to the aforementioned SFE process, the high selectivity of SFC enables the industrial production of up to 99% pure individual fatty acids such as EPA-99%. In fact the only two technologies that are capable of producing, by themselves, a EPA-99% starting from a basic fish oil ethyl ester are SFC and LC (HPLC). All other techniques separate either according to the chain length (molecular weight, boiling point, melting point) or to the degree of unsaturation. Only LC and SFC separate according to both chain length of the fatty acids and number of double bonds.

LIQUID CHROMATOGRAPHY

seen by the fact that the isolated fatty acid fraction obtained from the separation column is diluted in huge amounts of organic solvents (mobile phase). These large volumes of often toxic organic solvents have to be removed quantitatively from the isolated fatty acid. This is not only expensive but is often the cause of oxidative stress for the polyunsaturated fatty acids. A further drawback of LC is that there always remains a risk that the final product may still contain traces of undesired organic solvents.

Nevertheless, LC techniques have been used for many years, mostly in combination with other pre-concentration processes like urea fractionation, crystallization, or MD to produce very highly concentrated omega-3 products.

MOLECULAR DISTILLATION

The process of concentrating individual fatty acids from fish oil with the help of vacuum distillation has been known for more than a century. (21, 22) Former et al. reported isolating a 99% pure DHA-methyl ester with help of this technology in 1938. (23) However, the molecular vacuum distillation of those days is not comparable with the very gentle modern short path or MD of today. Whereas in former days the evaporated unsaturated fatty acid had to travel a rather long way to reach the condenser, and thus spend a long time under high temperatures, the distance between evaporator and condenser of a distillation unit in modern MD is extremely short. Consequently, the thermal stress on the sensitive polyunsaturated fatty acids is dramatically reduced. Modern MD is an efficient way to produce especially semi-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 if distilled under a strong vacuum (0.001 mbar) . Under these conditions, at temperatures between 140 and 170°C, a good separation between smaller C18 fatty acids and larger C18 fatty acids can be achieved, propelling the initial EPA and DHA content from 30% to approximately 50–60%. The classical standard MD concentrate on the market is a 33/22EE, or 33% EPA and 22% DHA; together, EPA and DHA total 55%. This is also the most popular oil found in concentrated omega-3 supplements. To a certain extent, it is possible to increase the EPA and/or DHA concentration with this technology. However, the price you pay is a substantial loss in yield. Passing a 33/20EE another time over the MD plant will result in a product having approximately 40–45% EPA with 10–15% DHA. Another pass over the MD could make a 50–55% EPA, and a subsequent pass could achieve an EPA 65–70% EE. Producing EPA or DHA concentrates with more than 70% of each individual fatty acid only with MD is, under normal circumstances, not possible owing to the lack of selectivity.

Table 19.1 presents a comparison of most common omega-3 concentration techniques.

MD separates only according to the different boiling points of substances in a mixture; in other words, it separates mainly according to the chain length of the individual fatty acids in the mixture. The degree of unsaturation has practically no influence on the separation. A mixture of highly unsaturated, long-chain fatty acid ethyl esters or their FFA is only separated according to their chain length. Consequently, this technique is capable of separating the C20 group from the C18 and C22 groups, but within the C20 the limitations of this technology.

CONCLUSION

This chapter gives a simplified overview on the most common omega-3 concentration techniques starting from the fishing vessel up to the final concentration processes. Most EPA and DHA concentrates found today on the market are produced by MD or SFC.

Table 19.1
Comparison of most common omega-3 concentration techniques

	MD	*SFC*	*LC*	*SFE*	*Urea*	*Crystallization*
Selective towards	Chain length (boiling point)	Chain length and C=C	Chain length and C=C	Chain length	Saturated fats	Melting point (Chain Length and C=C)
Operation temperature	140–220°C	35–50°C	20–50°C	35–50°C	−10 to 90°C	0 to (−70°C)
Operating pressure	0.001 mbar	>140 bar	1 bar	>140 bar	1 bar	1 bar
Use of toxic solvents	No	No	Possible	No	No	Possible
Max EPA/DHA concentration achievable (without any other technology applied)	65–75%	99%	99%	75–85%	45–65%	>90%
Decontamination efficacy	Very high	Very high	High	Medium	Low	Low
Mode of operation	Continuous	Semi-continuous	Semi-continuous	Contin-uous	Batch	Batch
Risk of product oxidation	Low	Very low	Possible	Very low	Possible	Possible
Flexibility to adjust EPA/DHA composition of final product	Limited	Very high	Very high	Limited	Low	Limited
Capital investment	Low	High	High	High	Low	Low

Table 19.2
Main fatty acids found in fish oils, grouped according their chain length

Chain Length	*C14*	*C16*	*C18*	*C20*	*C22*
Fatty Acids	C14:0	C16:0	C18:0	C20:0	C22:0
	C14:1	C16:1	C18:2	C20:1	C22:1
			C18:3	C20:4n6	C22:5n6
			C18:4n3	C20:4n3	C22:5n3
				C20:5n3	C22:6n3
				(C21:5n3)	(C21:5n3)

Even if a particular separation technique could isolate the C20 from the C18 and C22 groups, no pure 100% EPA could be expected. This is only possible by applying a second separation technique that separates according to the number of double bonds of the fatty acids

MD is also an excellent technology to decontaminate, (24–27) remove cholesterol from fish oil triglycerides, (28) and, as known for many years, to concentrate fatty acids or their esters. (29) 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 required for the MD is substantially lower than the investment required for an industrial SFE and SFC plant. Both technologies claim to be proprietary and are covered by patents in many countries. (18–20, 24–28) The only difference between these two technologies is that SFC can produce more highly concentrated products, and fish oil passing through the MD process encounters up to 350% higher thermal stress compared to a product passing through the SFE/SFC technology. However, so far no scientific proof has been given that products from MD contain more thermal decomposition products than SFC products. The undeniable increased thermal stress for EPA and DHA concentrates manufactured by MD may have an impact on their stability and, thus, shelf-life, but this still has to be proven.

MD seems to have economic advantages in the lower to middle range concentrates going up to an EPA+DHA concentration of about 50–70%. 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 highly concentrated products, the selective and very gentle SFC technology is the method of choice.

As mentioned in this chapter, most of the technologies discussed are not able to produce EPA and DHA concentrations of more than 85–90%, if applied on their own. They are either selective towards the chain length or towards the number of double bonds in a fatty acid molecule. However, in fish oil there are several different fatty acids with differing numbers of double bonds and all with the same chain length of the molecule. This is especially the case for the the C20 and C22 groups. Therefore, a combination of different technologies and, thus, selectivities, is always useful and recommended to manufacture highly concentrated EPA and DHA products.

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