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REVIEW

New perspectives of omega-3 fatty acids from diatoms

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Abstract

Omega-3 fatty acids are polyunsaturated fatty acids that are vital for human food consumption and metabolism. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two long-chain polyunsaturated fatty acids (LC-PUFAs), are primarily obtained from diatoms in the oceanic food web. Though microalgae are the main producers of EPA and DHA, but currently, only few algal strains are known to produce large levels of EPA and DHA. The demand for nutraceuticals has significantly increased because of people's increased awareness and health consciousness. Due to foods being the concentrated supply of omega-3 PUFAs (polyunsaturated fatty acids), this has increased the demands on aquatic sources of n-3 PUFAs. Micro-algal sources must be carefully examined due to the numerous drawbacks and difficulties of fish oils and the lack of DHA and EPA in plant sources. This review focuses on the current state of omega-3 PUFA (polyunsaturated fatty acids) production, sources, and market demand to provide an overview of sources that are being explored for sustainability as well as current and anticipated market trends in the omega-3 industry. This will make it possible for them to be produced on a wide scale for the benefit of human health.

Keywords Diatoms · Docosahexaenoic acid · Eicosapentaenoic acid · Nutraceuticals · Omega-3 fatty acid

Introduction

Diet plays a vital role in maintaining health and has a noteworthy impact on it; as a result, a poor diet is one of the major factors determining global morbidity and fatality due to nosocomial illnesses [3]. In developing and underdeveloped countries, the lack of proper nutritional diet for the maximum population is not available, which leads to various health conditions, such as cardiovascular diseases and neurodegenerative diseases. As this disease is very much related to the nutritional habits of individuals, distinct types of nutritional supplements containing important bioactive molecules can be of significant use to tackle such health conditions. Recent breakthroughs in the fields of nutraceuticals and nutritional biotechnology have unfurled new aspects of bioactive molecule supplementation in the diet [66, 78, 84, 106]. The n-3 fatty acids are the molecules that have been found to be beneficial in the treatment of neurological disorders, cardiovascular diseases, particularly those involving the coronary system, various cancers, and inflammatory conditions that arise because of various diseases [78]. Several kinds of microalgae have lipid contents that can make up 20–50% of the dry weight of the cell. Consuming DHA and EPA can lower triglyceride levels and lower your risk of developing cardiovascular disease. In addition, these fatty acids have positive impacts on fetal development and anti-inflammatory qualities. Currently, fish oil is the main supplement source for omega-3s. Although this product has health advantages, overfishing endangers fish species and the aquatic habitat. Additionally, the endocrinological system of consumers may be adversely affected by hormones used in pisciculture and hazardous metals including persistent organic chemicals that bioaccumulate in fish oil. This makes oil made from diatoms an alternative to meet the requirement for omega-3 in the human diet [105].

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Docosahexaenoic acid (DHA, C22:6) and Eicosapentaenoic acid (EPA, C20:5) are two important and essential n-3 polyunsaturated fatty acids (PUFAs). DHA and EPA are called essential as these are not produced in enormous amounts in humans. Plant wellsprings of n-3 PUFAs, for example flaxseed, known for the highest concentration of plant omega-3 fatty acids do not deliver EPA and DHA; rather, they produce more limited chain n-3 PUFA, α-linolenic acid (ALA). ALA does not present the well-being properties related to EPA and DHA, despite the former being an omega-3 unsaturated fatty acid [106]. Micro-algal sources including diatoms and other photosynthetic organisms, such as phytoplankton, are critical hotspots for n-3 EPA and DHA production. The expanding interest for bioactive compounds as the key molecules in increased demand of DHA and EPA in nutraceuticals features the need to recognize elective supportable wellsprings of n-3 LC-PUFAs. There is immense need for identification of economical elective bio-sources and opting the biotechnological techniques to improve and form stable sources and methodology of production of EPA and DHA by diatoms [46]. The n-3 PUFAs, EPA, and DHA are known as very long-chain omega-3 fatty acids making up a modest fraction of daily dietary fat consumption and can be present in two main food sources: plants and fish. The major plant omega-3 fatty acid ALA majorly found in flaxseed acts as a precursor to EPA and DHA [5, 74]. However, the biotransformation of ALA to EPA and DHA in the human body is extremely poor. Most fatty fish, such as albacore tuna, provide a rich source of both EPA and DHA. The most abundant primary sources of EPA and DHA are the marine microalgae *Nannochloropsis, Phaeodactylum, Schizochytriu,* and *Thraustochytrium* are just a few of the autotrophically and heterotrophically cultivated microalgae that have been studied for their high EPA and/or DHA content [87]. Varied physiological situations cause different fatty acid quantities to be generated [73]. The accumulation of generated fatty acids is strongly linked to microalgae growth stages, serving as an energy reserve during adversity or cell division. Due to their superior flow characteristics, EPA and DHA are primarily produced as acyl components of structural lipids, which are critical for cellular membrane operations. Nitrogen is imperative to diatom's legitimate physiological capacities, and it is a vital part in digestion of amino acids, nucleic acids, and photosynthetic shades and its take-up is at the most elevated level among all supplements [117].

Diatoms are a promising oil feedstock and energy source as they collect a lot of lipids comprising of Triacylglycerols (TAGs) and various unsaturated fats. Numerous significant lipids and lipid subordinates like fundamental unsaturated fats, steroids, and oxylipins can be delivered in diatoms in normal development climate. Algal oils enjoy upper hands over fish oils for being a veggie lover or vegan diet, since they do not contain any creature items. Also, being harmless to the ecosystem, algal oils are a solid substitute for fish oils due to diminishing fish stocks [43, 76]. Diatom-determined algal oils, as a substantial group of green growth, may be popular and receive a lot of attention in the global market because of their potential for production in comparison to fish oils [79]. At present, the primary dietary source of both the essential fatty acids, EPA and DHA, is marine fish population. Consumption of wild fish, contamination of the marine environment, and extension of the hydroponics business are factors which direct the need of new sources of EPA and DHA. Microalgae are the primary producers of LC-PUFAs, providing a consistent and potential alternative to fish oils. However, producing high-value products such as EPA and DHA from microalgae is expensive, and major efforts in strain research and development improvements are still necessary to minimize the existing high production costs associated with algal biomass. Also, the interest in delivering DHA and EPA of microbial sources as a choice to replace fish oil sources has increased in the last couple of years (see Table 1).

Sources of omega-3 fatty acids

Omega-3 fatty acids can be found in a variety of organisms, including animals, transgenic plants, and several microbes, including microalgae, but are mostly derived from fatty fish [1, 18, 42]. Naturally, ALA is found in plant oils, whereas DHA and EPA are found in fish and other Marine foods. Dietary supplements containing EPA and DHA are in high demand due to their numerous health advantages [30]. Fish, being the richest source of n-3 PUFAs, is considered as

Table 1 Poly-unsaturated fatty acid found in diatoms

S. no	Diatom	EPA %	DHA %	PUFA %	MUFA %	References
1	*Halamphora coffeaeformis*	9.40	2.56	36	32.3	[91]
2	*Navicula incerta*	15.97	0.19	23.25	45.34	[39]
3	*Amphora copulata*	3.6	–	10.6	44.5	[54]
4	*Nitzschia sigma*	16.55	1.69	38.62	26.49	[39]
5	*P. tricornutum*	4.0–9.1	9.5–13	31.41	21.91	[58]
6	*Proschkinia luticola*	12.69	1.61	39.04	39.43	[39]

the best and most easily available source. This is because many fish devour algae high in EPA and DHA, as do other organisms that consume algae, such as fish and marine invertebrates [36]. This resulted in a significant increase in fish demand (aquaculture sector growth) and put immense stress on dwindling population of marine species. However, due to pollution in the aquatic ecosystem, researchers have turned their attention to another promising supply of n-3 PUFAs. Furthermore, vegetarians do avoid the long-chain n-3 PUFAs from animal sources. At present, EPA- and DHA-rich plant seeds, thraustochytrids, and microalgae are currently being explored for commercial synthesis of vegan n-3 PUFAs [31].

The traditional and most well-known source of n-3 PUFAs is fish, which contains elevated levels of EPA and DHA. In addition to the load on the marine ecosystem as discussed earlier, it can cause various health conditions in humans as the marine fishes can contain several environmental toxins, such as mercury, polychlorinated biphenyls, chlordane, dioxins, and dichlorodiphenyltrichloroethane (DDT) [65]. Out of these, mercury is a serious point of concern as it is not available freely in the environment and gets converted into methyl mercury—the organic form of mercury and enters the food chain after it is taken up by marine animals [65]. Thus, alternative plant and microbial sources are considered, and more research is emphasized on these sources [20].

The most prevalent source of fatty acids in humans is the intake of vegetable oils. Sunflower, palm, and soybean oil dominate the vegetable oil market. The primary components of palm oil are oleic acid (43%), palmitic acid (40%), and linoleic acid (11%) with exceptionally low amounts of n-3 ALA [44]. Flaxseed is the main plant source of n-3 PUFA with the lowest concentrations of saturated fatty acids (SFAs). The only limitation associated with flaxseed oils is that it has an extremely high oxidative nature and cannot be used for cooking purposes. Flaxseed oil is used as nutraceutical and functional food [56]. *Camelina sativa* is another minor oilseed crop that originated in ancient times bearing high concentration of ALA [113]. *Ocimumbasilicum*, grown in the regions of Asia, Africa, and Central and South America's tropical regions, has great therapeutic and aromatic vale. Basil seeds are also reported to contain ALA up to 57–71% [85].

Microalgae are the principle source of PUFAs in marine water and play a key role in their primary production. Some marine invertebrates can also manufacture certain n-3 PUFAs de novo; thus, they are also considered as the important primary sources of omega-3 PUFAs [36]. For many years, researchers have been looking at the possibility of using microalgae as a source of Omega-3 fatty acids. Diatoms are types of algae that lead to the production of elevated levels of n-3 PUFAs and have an important part in the world climate change and ecosystem dynamics [16, 41]. Thraustochytrids are a valuable source of dietary EPA and DHA in the industrial sector. They are a *Stramenopile* heterotrophic fungus-like group. Vegan EPA and DHA are commonly produced commercially using Thraustochytrids, particularly *Schizochytriumm, Aurantiochytrium, Crypthecodinium*, and *Ulkenia* species [77, 89, 94]. Some *Schizochytriumm* sp. can accrue substantial amounts of EPA and DHA (16.18 and 33.72%, respectively), whereas DHA is distinctively deposited across most *Schizochytriumm* sp. (37.10–63.1%) [17, 57], *Aurantiochytrium* (30–40%) [62], *Crypthecodinium* (40–45%) [99], and *Ulkenia sp.* (45%) with traces of EPA [67]. Figure 1 represents various sources of n-3 fatty acids that help in enhancing IQ levels in children, help reduce inflammation, and reduce the risk of cardiovascular diseases which is further discussed in the importance of fatty acids.

Extraction and purification of lipids

Microalgae are currently being explored as potential nutraceuticals. The fatty acids generated by the microalgae are the major focus. As a result, techniques for extraction and purification of fatty acids found in microalgae are required. Bligh and Dyer devised an extraction technique based on chloroform and methanol in 1959, which proved to be an effective solvent combination for penetrating cells and recovering total lipids from fish tissue [23]. For fatty acid extraction, this approach is commonly used and has been cited more than 60,000 times. Although Bligh and Dyer extraction is frequently used in microalgae, due to changes in cell wall and lipid content, chloroform–methanol may not be the best solvent system for lipid extraction. Furthermore, the Bligh and Dyer technique was originally claimed as being particularly effective for low-lipid tissue samples [14]. Xia et al. [96] reported another method for the extraction of total lipid from microalgae. For the total lipids extracted from microalgal biomass, concentration depends heavily on the solvent or combination used. The solvent mixture of chloroform–methanol in 1:1 concentration yielded the maximum lipids, making it ideal solvent for the determination of total lipids. Lyophilized microalgal biomass can be used for this analysis, which makes weighing easier and more accurate. This approach does not require pretreatment with isopropanol or any other solvent to inactivate the lipases or the addition of antioxidants. Organic solvents, such as dichloromethane, hexane, methanol, chloroform, ethanol, and petroleum ether, have been commonly utilized to extract lipids from microalgae due to their inexpensive cost [47]. Although the extraction approach using supercritical fluid enhances lipid extraction using less hazardous solvents, it has a significant operating cost. Combining different techniques

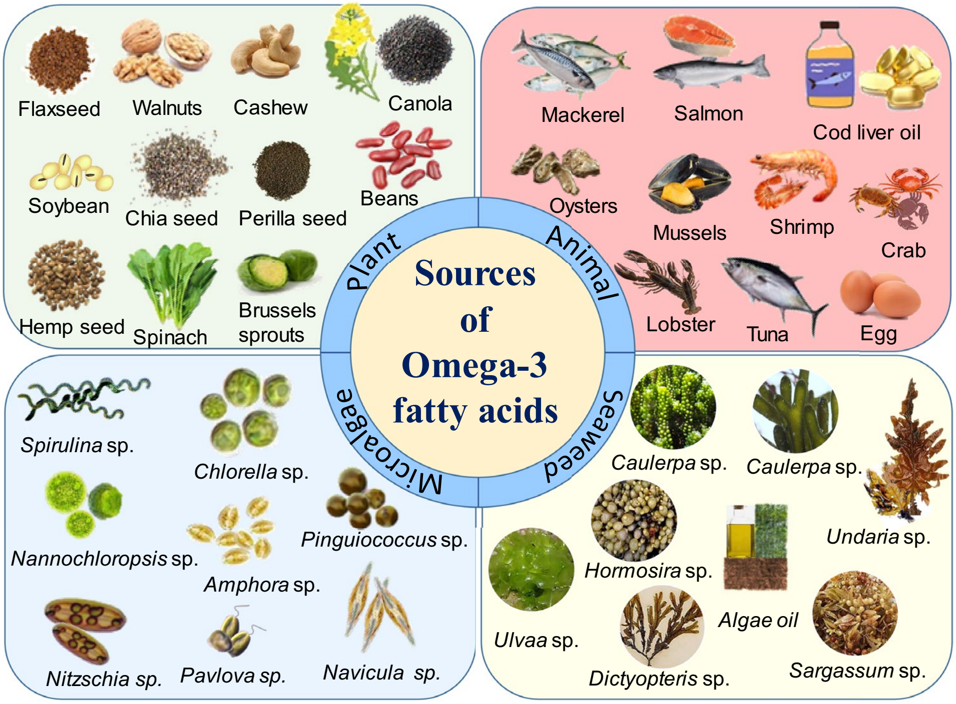

Fig. 1 Various sources of omega-3 fatty acids. There are four main sources of omega—3 fatty acids—plant (flaxseed, walnuts, soybean, etc.), animal (Salmon, Tuna, Eggs, Shrimp, etc.), microalgae (*Spirulina sp., Chorella sp, Pavlova sp*., etc.), and seaweed (*Caulerpa sp., Undaria sp.,Ulvaa sp*., etc.)

for the extraction and purification of EPA and DHA from microalgae can result in efficient yield of the desired product [121]. Saxena and Kumar [100] introduced a new efficient and environmentally friendly method for extraction of total lipids from algae. The highest total lipid yield from algal biomass (32.8 percent on a dry basis) was obtained using an Ethanol/Cyclopentyl Methyl Ether (6:4) mixture at 80 °C incubated for 60 min. These extraction conditions are good for obtaining oil high in EPA and DHA, both of which contribute to human well-being. To increase the extraction rate of microalgae lipids, supercritical carbon dioxide (SC-CO_2) can be utilized as an alternative to organic solvent extraction. Oliver et al. [86] used SC-CO_2 with ethanol as a co-solvent to extract neutral lipids from freeze-dried *Nannochloropsis oculata* and *Chlorella vulgaris*. Under optimal conditions, the lipid extraction rates of *Nannochloropsis oculata* and *Chlorella vulgaris* were 83 and 97%, respectively. Vollmann et al. [112] improved the SC-CO_2 lipid extraction method from microalgae (*Chlorella protothecoides*), with the highest lipid extraction rate recorded at 300 bars and 70 °C. SC-CO_2 changes the fatty acid composition of the extract while also increasing the extraction rate. Evaluation of the extraction of lipids containing PUFAs from *Nannochloropsis oculata*, a marine species rich in EPA, using a commercially available pressurized fluid extraction technique (PFE, also known as accelerated solvent extraction) [90]. Ethanol extraction yielded the maximum yield (36 ± 4 mass percent), whereas n-hexane extraction yielded the lowest yield (6.1 ± 0.3 mass percent). The biomass extracted using ethanol produced the highest yield of fatty acids up to 16.7 ± 0.6. Many techniques have been reported for extraction of lipids from microalgal biomass. By dielectrically warming the solution of water-based (as a polar solvent) during microwave-assisted extraction, which produces vapor within the tissues along with causes modifications to their molecular makeup such as breakage that results in electroporation, the cell membrane is opened up, causing the dissolution of inside of cells substances [78, 79]. It has been demonstrated to be a quick, safe, and effective method when used with wet microalga biomass. To boost extraction yields, ultrasound might be implemented with other methods. They looked at a liquid biphasic flotation-supported sugaring-out extraction method along with protein removal using ultrasound. However, ultrasound-assisted extraction maintains the integrity of the fatty acids and, as a green method, avoids the use of solvents while speeding up the recovery of the oil. One of the earliest and most basic techniques for obtaining oil from oil seeds is the expeller press, often known as an oil press. The idea behind this method is to squeeze the oil out of the algal biomass by applying high mechanical pressure to shatter and crush the cells. The concept of spinning the biomass slurry at high speed with tiny beads causes direct damage to the cells during bead beating. Bead beating is an industrial technique for the disruption of cells. The effect of crushing beads against cells occurs in bead mills, disrupting the cells. The technique can be used to handle any sort of cell, including those from microalgae. Bead mills typically come in two different varieties: shaking containers and agitated beads. An industry initiative by OriginOil, which has created a method that does not need organic solvents for algae oil extraction, is one such attempt that is deserving of note here. Instead, it employs brief bursts of low-power, frequency-tuned microwave energy to shatter the intricate and tough algal cell walls. The oil extraction is then quickly finished using quantum fracturing on the now-precracked cells. According to the OriginOil Corporation, this distinctive technology enables low-energy and environmentally safe algal oil manufacturing [78, 79]. The invention (patent no. US5244921) relates to growing microorganisms in an air lift fermenter, causing those microorganisms to produce edible oils rich in omega-3 fatty acids, and recovering those oils and/or fatty acids. The invention also relates to diatoms having higher levels of omega-3 fatty acids than wild-type diatoms growing in the wild, mutant diatoms, and novel oils containing omega-3 fatty acids but lacking the extra polyunsaturated fatty acids associated with fish oils. These oils typically go on to be described as having biphasic melting patterns. It demonstrates how to separate food-grade oils from organic matter. The result is a biomass slurry made up of microbial particles suspended in water. Usually, the slurry is spun in a centrifuge before being homogenized. The resultant slurry is then combined with an organic solvent that is virtually immiscible in water, such as hexane, and supplied onto a contacting device, like a packed column. The solvent separates the oil from the biomass mixture after extracting it. The solvent is collected and used to further refine the edible oil (Patent no.US6166231A).

Biosynthesis of unsaturated fats and neutral lipids in diatoms

Marine diatoms are fantastic producers of exceptionally significant polyunsaturated fatty acids like EPA and DHA. Subsequently, it is specifically compelling to explore their biosynthetic pathways, which could be additionally changed and streamlined for mechanical creation [58]. Figure 2 shows the biosynthesis pathway of long-chain unsaturated fatty acid responses. In this pathway, Linoleic acid (18:2) is converted into stearidonic acid using desaturase enzyme which further forms ecosatertraeonic acid in the presence of elongase [15, 48]. The ecosatertraeonic acid is further converted into EPA by the action of δ-5 desaturase. Then, EPA gets converted into DPA with the help of elongase which further undergoes elongation in the presence of elongase, forming tetracosapentaenoic acid. Then, this further gets converted into tetracosahexanoic acid by δ-6 desaturase. In the last step, tetracosahexanoic acid undergoes β-oxidation

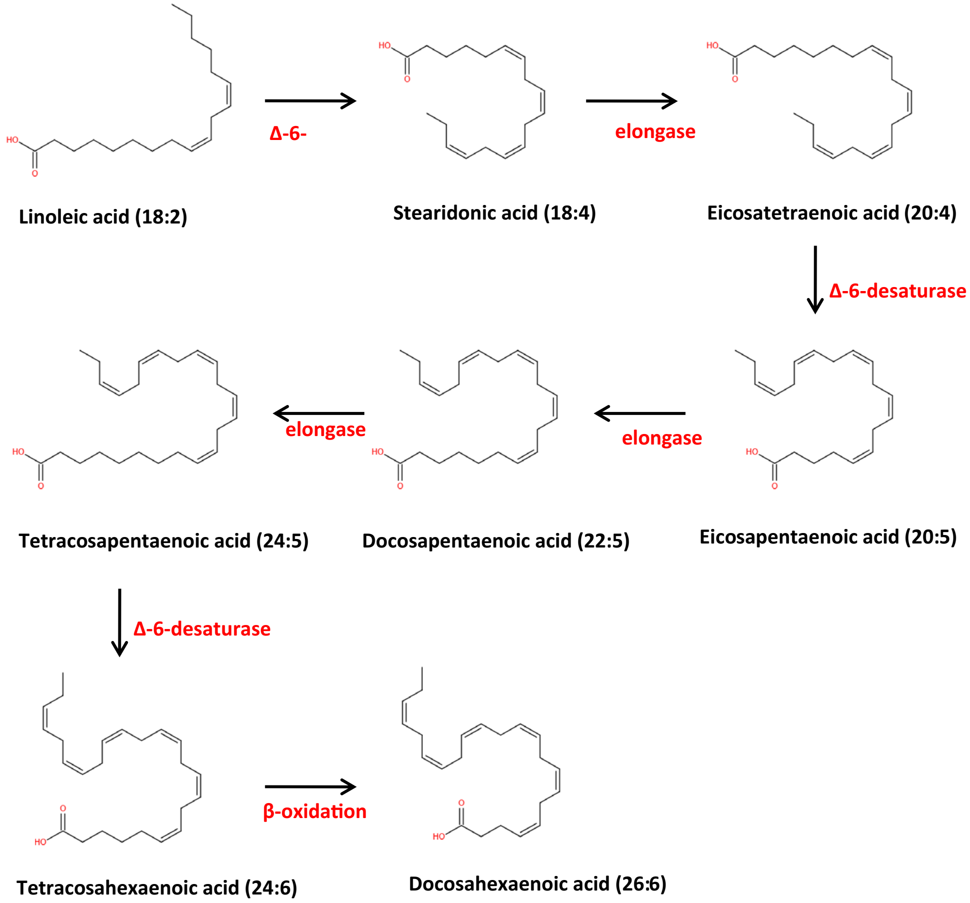

Fig. 2 Biosynthetic pathway of EPA and DHA in diatoms. In this pathway, linoleic acid (C18:2) is converted into stearidonic acid using desaturase enzyme which further forms eicosatetraenoic acid in the presence of elongase. The ecosatertraeonic acid is further converted into EPA by the action of δ-5 desaturase. Then, EPA gets converted into DPA with the help of elongase which further undergoes elongation in the presence of elongase, forming tetracosapentaenoic acid. Then, this further gets converted into tetracosahexanoic acid by δ-6 desaturase. In the final step, tetracosahexanoic acid undergoes β-oxidation to form the final product of the biosynthetic pathway, i.e., DHA. Biosynthesis pathway of long-chain unsaturated fatty acid responses

to form the final product of the biosynthetic pathway, i.e., DHA. Different metabolic processes are used by aquatic eukaryotes with one cell to produce PUFAs. The most well-known traditional method is O2-dependent and begins with the FAS pathway, which enables the synthesis of fatty acids from acetyl-CoA and malonyl-CoA [13, 40]. To produce long-chain polyunsaturated fatty acids, it is carried out by extension and the desaturation activities of the n-3 and n-6 pathways. The alternate-3 desaturase pathway can link these two processes. In Haptophytes microalgae like *Isochrysis galbana* and *Pavlova salina*, another pathway involving 8 desaturases has also been discovered [97]. This mechanism avoids the 6 desaturation of the n-3 and n-6 processes. In heterotrophic protists like thraustochytrids, the polyketide synthase route (PKS pathway), which generates C20:5n-3 and C22:6n-3, is present. Even while certain microalgae species share some or all these synthesis routes, primary producers' PUFA composition differs widely between species. For instance, dinophytes are higher in C22:6n-3 than C20:6n-3, but diatoms are richer in C20:5n-3 than C22:6n-3. Comparatively, EPA and DHA are either non-existent or exist in extremely lesser amounts [less than 1% of the total fatty acid (FA)] in cyanobacteria and some chlorophytes phyla. Similar fatty acid profiles were found in C. muelleri and other diatoms, with PUFA being more prevalent in polar lipids and SFA and MUFA being more prevalent in neutral lipids. The primary fatty acids for polar and neutral lipids were C14:0, C16:0, C16:1n-7, and C20:5n-3 (EPA). The significant concentration of 16:1n-7 and its substantial enrichment backed up its essential function as a C16 PUFAs pathway precursor. C16:1n-7, which is first desaturated in C16:2n-7 or C16:2n-4, initiates the C16 PUFAs route. The desaturation of C16:2n-4 then occurs once more in 16:3n-4 and ultimately in 16:4n-1. This suggests that the diatom mostly used the n-3 pathway to synthesize C20:5n-3 rather than the n-6 pathway. Furthermore, since these two FA were only present in trace levels, it is likely that the alternative 8-desaturase route in *C. muelleri* was limited. This process converts C18:3n-3 into C20:3n-3 and then into C20:4n-3. The production of C18:4n-3 and C20:5n-3 via 3'-desaturation of C18:3n-6 and C20:4n-6, respectively, seems more feasible. The two most significant n-6 PUFAs were C18:3 and C20:4 n-6 fatty acids [93].

The transformation of a metabolic route to enhance one or more attributes is known as metabolic engineering. It is necessary to comprehend the metabolic processes that regulate the breakdown of lipids to improve the generation of omega-3 fatty acids in microalgae. To increase the production of omega-3 fatty acids, efforts have been undertaken to target the omega-3 fatty acid synthesis route, the availability of precursors, and transcription factors. Fatty acid biosynthesis begins with the carboxylation of acetyl CoA to malonyl CoA, followed by the creation of a protein called a malonyl-acyl carrier protein (ACP) that is catalyzed by the MCAT gene. Algal strains have been screened, biosynthetic pathways have been explored, induction settings have been optimized, and algae-cultivation methods have been improved in recent years to promote the commercial manufacturing of microalgae polyunsaturated fatty acids. Temperature, light, salt content, and carbon supply all have a direct impact on the creation of phytoplankton polyunsaturated fatty acids. A successful strategy for growing microalgae that are high in polyunsaturated fatty acids is two-stage culture. Additionally, it has been shown to be a promising strategy to encourage the sustainable growth of human society to use algae-derived omega-3 fatty acids in livestock feed [26, 10].

Genetic modifications for ω-3 fatty acid

In a study by [59], *P. tricornutum* was genetically modified to accumulate higher quantities of by overexpressing heterologous genes that code for DHA and DPA LC-PUFA biosynthesis pathway enzyme activity. It was reported that the simultaneous expression of OtElo5 and OtD6 revealed further increases in DHA levels, marking the first time in a transgenic diatom that two heterologous enzyme activities were co-expressed [25]. Transgenic co-expression of a 4-desaturase with OtElo5 5-elongase activity is likely to result in even higher concentrations of DHA, because endogenous 4-desaturation is not a significant bottleneck in the synthesis of DHA (via transgene-derived DPA). The microalgae *N. oceanica* CY2 was engineered and it was observed that EPA buildup and microalgal development are strongly influenced by the composition of the medium, the concentration of the nitrogen source, and the light sources [33]. The most effective EPA generation was placed when the microalgae were cultivated in altered BG11 medium at a $NaNO_3$ concentration of 1.50 g/L with LED-blue as the light source, the most efficient EPA production occurred, with an optimal EPA content (5.57%) and EPA productivity (12.29 mg/l/day), highlighting the great potential of utilizing this technique to produce EPA for use in industry from microalgae.

Importance of n-3 PUFAs

Nutraceutical importance

More than 30 years ago, the term “nutraceutical” was coined [24]. Among the several classifications, González-Sarrías et al. [53] defined nutraceuticals as “a sort of dietary supplement that supplies a concentrated form of a putative bioactive agents, nutritional or non-nutrient, but from food source." Omega-3 fatty acids are the family of fatty acids which cannot be synthesized by the human body, making them "essential" and requiring dietary supplementation. Chronic omega-3 administration improves reference memory and learning, as well as neuron membrane neuroplasticity [22, 50]. n-3 PUFA reported to inhibit ovarian cancer by elevating the TGF-1, Smad-3, and p21 levels [103]. In an animal model of atopic dermatitis, DHA increased TGF-1 expression and suppressed the release of pro-inflammatory cytokines through CD4 + T cells and macrophages, demonstrating immunomodulatory and anti-inflammatory properties [7, 61]. Aged retinal pigment epithelial cells (ARPE) are found to have impaired autophagy. Nutraceuticals containing n-3 fatty acids in combination with resveratrol were reported to have positive homeostatic effects in RPE cells and protect these cells from the damage [68]. DHA treatment to d-galactose-induced aging mice reduced oxidative stress, DNA damage, and the cellular aging cascade [34]. Dietary supplementation with EPA and DHA has been shown to improve heart failure prognosis, particularly in individuals with myocardial infarction. This promotes integration of DHA into mitochondrial membrane phospholipids and lowers the susceptibility of segregated cardiac mitochondria to sustain mitochondrial permeability transition driven by Ca2 + and stress. DHA may reduce membrane viscosity, allowing membrane proteins to flow more easily [37]. Table 2 contains some of the commercially available n-3 PUFA around the world. For laying hens, dietary supplementation with supplies of EPA, DHA, or its antecedent is a popular method of enhancing eggs with desired n-3 PUFAs. The primary component for both omega-3 fatty acids is -linolenic acid, which has enzyme pathways to produce EPA and DHA. Many people supplement their diets with certain plant- and animal-derived foods to increase their intake of ALA, such as linseed canola, linseed, sunflower seeds, seafood oil, algae, etc [2]. However, according to several scientific studies, the conversion of ALA to EPA and DHA varied significantly depending on the FA sources, and the conversion to DHA was frequently constrained by the distinct desaturation and elongation pathways. For instance, adding extruded flaxseed to meals up to 9% resulted in eggs containing 150 mg DHA and 530 to 670 mg n-3 PUFAs, while adding *Aurantiochytrium limacinum* microalgae to diets at 1% might result in eggs containing 286 mg DHA and 626 mg n-3 PUFAs. Depending on the source, dosage, and type of supplemental fatty acids, reports on the DHA and n-3 PUFA contents of eggs utilizing fish oil, microalgae, linseed oil, sunflower oil, and combinations from other sources ranged between 50 and 290 mg/eggs [28, 6]. Today, marine fish oils are the primary source of n-3 LC-PUFA from aquatic life used in human diet. Fish typically receive EPA through the absorption through their nutritional chain, whereas microalgae produce n-3 LC-PUFA, which raises their receptivity to contamination by contaminants like heavy metals. To address the increasing need for vegan n-3 LC-PUFA, it has become necessary to look for substitute sources of energy due to the pungent smell of the oil that is obtained and the loss of fish resources [27]. Although producing n-3 LC-PUFA by an autotrophic alga is theoretically viable, there are still many obstacles to overcome before it can be done so profitably. An uncommon instance of an autotrophic alga that can collect significant levels of TAG containing n-6 LC-PUFA is the freshwater chlorophyte *Parietochloris incisa.* According to reports, a small number of additional taxa (such as *Pavlova lutheri, Nannochloropsis oculata)* also exhibit a reduced degree of LC-PUFA partitioning to TAG [9].

Therapeutic importance

EPA and DHA, as well as other n-3 PUFAs, have been reported to help reduce the progression of age-related disorders like cardiovascular disease, inflammatory conditions, and Parkinson's disorder in a few epidemiological, clinical, and pre-clinical studies [38, 45, 64, 110, 109, 118]

Cardioprotective impact

Cardiovascular infection (CVD) is the significant reason prompting passing in numerous nations. This is because

Table 2 Commercially available n-3 PUFAS

S. no	Commercial brand	Source	Product	References
1	Solutex GC	Algae and Fish	Oil	[52]
2	Aker BioMarine	Krill	Oil	[21]
3	Croda international PLC	Fish	Oil	[116]
4	Omega protein corporation	Fish	Oils	[102]
5	Pharma marine	Fish	Oils and concentrates	[51]
6	GC rieber	Fish	Oil	[51]
7	Clover corporation	Fish	Oils, powders and micro-encapsulations	[105]
8	Cellana	Algae	Oils	[32]

of the maximum usage of soaked fats that prompts CVD, while admission of advanced PUFAs shows bring down this causes. Studies have announced that with high fish utilization, there is low cardiovascular mortality among populaces as fish includes a significant degree of omega-3-unsaturated fat (EPA and DHA). Studies have likewise revealed that omega-3-unsaturated fat, for the most part long-chain (LC) unsaturated fat, likewise secures against CVD by diminishing the heart defenselessness to arrhythmias, bringing down circulatory strain, and so on [35, 49]. The preliminary that was accounted for to be the main preliminary to point omega-3-unsaturated fat to forestall coronary illness was Diet and Re-infarction preliminary. It is accounted for that supplementation of 1 g/day omega-3-unsaturated fat for about 3.5 years decreased fatty oil level and hazard of CVD. In this manner, it is encouraged to devour more fish and fish items [106].

Parkinsonian impact

The infection comprises resting quakes, postural unsteadiness, bradykinesia, and strong unbending nature. Omega-3-unsaturated fats and Parkinson's infection (PD) are connected, so that enhancements of omega-3-unsaturated fats help control qualities engaged with mind irritation and body digestion in patients managing Parkinson's infection [70]. The utilization of omega-3- unsaturated fats and Vitamin E assists with controlling neurological side effects in PD patients. These two enhancements additionally help increment the peroxisome multiplication activator receptor gamma quality action, which is a quality associated with lipid and insulin digestion [11].

Anti-inflammatory effects

Omega-3-unsaturated fats contain pro-inflammatory leukotrienes which are framed when arachidonic corrosive (AA) changes over with the assistance of proteins called cyclooxygenase (COX). As omega-3-unsaturated fat contains EPA and DHA, they go about as a substrate for COX. Hence, it is concentrated on that the EPA and DHA assist with lessening irritation [106].

Fetal advancement improvement

These unsaturated fats are fundamental to keep up with equivalent or adjusted creation of chemicals like prostaglandin and so on. This chemical directs numerous physiological capacities like pulse, irritation, unfavorably susceptible reactions, and so forth [29, 60]. These unsaturated fats are additionally observed to be fundamental for the neurological and visual improvement of the baby. Regardless, during pregnancy, this unsaturated fat becomes inadequate in a lady's body, since the entirety of the omega-3-unsaturated fats is utilized by the baby for sensory system advancement. After pregnancy, this unsaturated fat is likewise used to make bosom milk. In this way, taking a suitable measure of omega-3-unsaturated fat during pregnancy decidedly influences the hatchling's visual and intellectual turn of events. This unsaturated fat likewise builds birth weight and lessens discouragement [106].

Limitations and challenges

Most common natural source of n-3 PUFA (DHA and EPA) is wild fish and there is a serious alarm of overfishing [63]. If current harvesting rates continue, global fish populations might be depleted in 40 years [71]. There are a few disadvantages of using fish as the diet's main source of EPA and DHA. Consumers must be informed of which fish species provide adequate quantities of EPA and DHA, because they are not present in high concentrations in all fish. Consumers must be aware of which fish have and do not contain certain contaminants, because fish is a nutritional source of mercury as well as other environmental toxins (polychlorinated biphenyls, dioxins) [55, 83]. Another common and primary source of n-3 PUFA is microalgae. Furthermore, there are species with low LC-PUFA concentration and thus low nutritive value among algal groups that synthesize EPA and/or DHA. Thus, there is a need for revision of the common point of view that all microalgae or diatoms are superior in synthesis of n-3 PUFA. Lipids and fatty acids, which serve as molecules for storing carbon, are abundant in diatoms. By 2018, the market for fatty acids was around $4.31 billion USD, and by 2026, it is projected to be worth 91.6 billion USD. Establishing a cost-effective cultivation approach to harvest the most diatoms in the shortest amount of time is an important feat in diatom research [8]. There are different extraction and purification procedures for n- PUFAs with respect to their source. Fish is considered as the most valuable ecological source of n-3 PUFAs in the nourishment, notably fatty fish oil, harvested on a huge scale using conventional methods since the nineteenth century processes based on few steps: boiling, decanting, pressing, and centrifuging fish. Alternative methods, such as those based on supercritical fluid extraction (SFE) and enzymatic procedures, are also being investigated [75]. The high yield (more than 96% in 3 h) of n-3 PUFAs is reported by supercritical CO_2 extraction of hake by-products from the fish industry. With high yield, the SFE method has a main limitation of production of high free fatty acids which increases the acidity of the extract [93]. For plant seeds, the most common techniques used for the extraction of n-3 PUFAs-rich oil are cold press extraction and solvent extraction. It has its own limitations of poor yield and health risks due to solvent residues left in the oil [19]. Some of the studies show the different solvent extraction methods of n-3 PUFAs from microalgae. They reported the highest yield in dichloromethane: methanol comparable to the chloroform:methanol. However, it is recommended for pharmaceutical use due to high regulations in food application. For edible applications, ultrasound probe-assisted ethanol extraction is recommended due to its lowest toxicity. The cost of production is one of the key constraints, because algae biodiesel cannot now compete with fossil fuels. The biomass from algal can be investigated as an alternate supply of dietary fiber when cost parity is attained [70]. Researchers and consumers are learning more about the significance of most dietary components in illness prevention and health promotion. Astaxanthin (carotenoid), which generates yellow to red pigments from *Haematococcus*, and phycocyanin, which belongs to phycobiliprotein and is blue pigments from *Spirulina*, are two examples of the various colors that these natural pigments produce. [81]. Consumers have the information they need to choose from a broad choice of foods products that either include health-promoting bioactive elements naturally or have those components added through fortification. The products with short shelf-life, such as bread, dairy, or frozen foods, have been fortified with high-quality fish oils. The limitation of this high-quality n-3 PUFAs-rich oil is in long shelf-life products that got readily oxidized during food processing and storage due to oxidation initiators and catalysts [108]. Fast, effective, and gentle extraction techniques should be used to prevent lipid or fatty acid breakdown. The extraction solvents ought to be cheap, volatile (for quick removal later), free of toxic or reactive contaminants (to prevent reaction with the lipids), able to form a dual-phase solution when combined with water (to eliminate non-lipids), and poor extractors of undesirable components (such as proteolipids, small molecules). It is important to think about how well the various kinds of lipids dissolve in various solvents. To extract very pure PUFAs from microalgae, a downstream method must yield large amount of product while not compromising the PUFAs' quality. The procedure includes the following steps: (i) centrifugation to concentrate the biomass from the culture medium; (ii) direct synthesis of wet biomass with KOH-ethanol to extract fatty acids; (iii) extraction of unsaponifiable; (iv) hexane to extract unsaponifiable; (v) urea to concentrate PUFAs; and (vi) preparative reverse phase HPLC to isolate the desired PUFA. The extraction and elimination of PUFAs using supercritical fluids is among other strategies that is quite promising, although further research is required to achieve higher selectivity. Solvent extraction is a typical method for recovering lipids from microalgae that is inexpensive, straightforward, and simple to use. Soxhlet extraction has been widely used to isolate bioactive compounds from solid samples of natural sources. Although this is a labor-intensive method that typically uses large amounts of solvent, it is appropriate for natural resources that have restricted absorption and enables reliable and independent extraction [13]. Supercritical fluid extraction (SFE), one of the other techniques, stands out as a green technology that may be used to get both the required omega fatty acids and the biodiesel lipids. Additionally, SFE has the potential to be carried out on an industrial scale, producing extracts devoid of potentially dangerous chemicals [91]. Although long-chain omega-3 fatty acids (EPA and DHA) are abundant in fish and marine sources, consumers are turning to alternatives to fish oil as the vegetarian diet becomes more popular. Depending upon the precise circumstances and the kind of contamination involved, contaminants' stressors like industrial waste or pharmaceutical waste can have detrimental impacts on the cultivation of microalgae and diatoms. There are several ways that pollution stress may affect the circumstances and output of microalgae and diatom cultivation. There are a few reports on the PUFA content of phytoplankton cultivated on effluent media; however, since EPA and DHA are membrane lipids, variations in pH, salinity, turbulence, and light have an impact on the fluidity and stability of membranes, this information is important. Because of this, effluent-grown diatoms could generate more PUFA throughout their hyperbolic stage, when they are continuously adjusting to dynamic changes in the physicochemical conditions of the wastewater [82]. Environmental factors like salinity, temperature, and pH may have an impact on the diatoms' and microalgae's growth and development. To maximize production, it is critical to keep focus on and manage these factors during cultivation. Due of their better ability for climate change, diatoms also have complicated mechanisms of metabolism, extensive chloroplast, and distinctive silica outer shells called frustules. Toxic pollutants, such as industrial chemicals, can enter the water because of pollution. Due to their sensitivity, such poisons can cause cellular damage and stunted growth in microalgae and diatoms. In extreme situations, it might potentially lead to the cultures' death and a drop in yield. In some circumstances, pollution can increase nutrient availability in the water, such as greater quantities of nitrogen and phosphorus from industrial discharge or agricultural runoff [82]. Nutrient-rich settings are ideal for microalgae and diatom growth, which can lead to faster rates of growth and larger yields when grown. Some diatoms and microalgae have evolved defenses against the stresses that exist in contaminated settings. They might predominate in contaminated waterways, because they have grown to endure larger concentrations of toxins. These modified strains could produce larger yields when grown in contaminated locations. Another point of concern for the consumption of n-3 PUFAs from marine sources is environmental toxins, such as mercury, polychlorinated biphenyls, chlordane, dioxins, and dichlorodiphenyltrichloroethane (DDT) [65]. Mercury is the lethal metal that is in the form of methyl mercury—the organic form of mercury, enters the food chain after it is taken up by marine animals [65]. Heavy metal exposure poses a major threat to aquatic systems. Diatoms are the most common phytoplankton that may detoxify heavy metals. Heavy metal ions can be effectively taken up and eliminated from the body by diatoms. As a long-term solution for heavy metal contamination, diatoms must be taken into consideration. Diatoms can display both-reactive and inappropriate phenotypic change in response to their environment. Teratological formations, which typically alter the shape or pattern of valves, are non-adaptive phenotypic aberrations. The most well-known causes of teratological variations embrace heavy metal contamination and artificial growth conditions. Cu, Cd, and Zn seem to be the small amounts of metals that strongly encourage the formation of abnormal cells. Tangled valves in *Nitzschia delicatissima*, a 90° rotation in the frustules of *Asterionella formosa*, and major deformities in A. japonica were all brought on by copper. In *Eunotia exigua, F. tenera, Fragilaria rumpens,* and *Eunotia sp*., it also resulted in abnormal valve outlines [32]. Infectious agents in some waste effluents, along with metals, could infect microalgae and pose safety risks. Consortia of pathogens could form in waste effluent and inoculated microalgae, posing a major hazard to the health of animals or people [4, 12].

Research needs and future directions

The entire manufacturing process, which depends on different micro- and macro-aspects like growth, harvesting, drying, and genetic variation as well as the absence of genomic, proteomic, and metabolic insights, is one of the primary obstacles in diatom-based industry. With advances in hereditary tools, fermentation techniques, and some structural upgrades, these problems may be overcome soon [24]. Academic research, industrialized research, structural, regulations, education, and information gaps are all obstacles in bio-based companies at various levels. Scientific research and innovations are progressing, as evidenced by publications, but they require help from other sectors, such as infrastructure development, bridging the knowledge gap between scientists and entrepreneurs, and changes in international regulations. To sum up, the recent research phenomena of diatoms' economic potential, which exploded in the last decade, nevertheless left many unanswered concerns. The main concern will be how much genetic or artificial alteration can be done without affecting both the silica pattern and its structure. Numerous limitations in genetic engineering, farming, and harvesting will be filled, enabling complicated plant pathways to be replicated in diatoms. These techniques have made it possible to investigate diatoms for environmentally beneficial operations [103].

Although the FDA has allowed using silica for food and agricultural applications, and the International Agency for Research on Cancer has labeled/classified it as non-carcinogenic, this could be a huge step toward advancing its usage in biomedicine. It has not yet been licensed for use in biomedicine, since enduring evidence is required [109]. Before they reach the level of commercial distribution, it is reviewed by numerous organizations with interest in the field of biomedicine, including researchers and government agencies like the province and federal governments. Given that it would be employed directly into the human, this is understandable. As a result, an advanced and unique method is necessary to convey in university investigators and bio-entrepreneurs to increase the level of enhancement in the biomedical organization without exposing the public health screening procedure. Entrepreneurs and researchers working together will be able to conduct a full analysis of the market for novel discoveries, industrialization, investment, and product globalization [103].

Conclusions

The most abundant primary sources of EPA and DHA are the marine microalgae. Omega-3 fatty acids are the family of fatty acids that the human body cannot synthesize, making them essential and requiring dietary supplementation. The main source of PUFAs is microalgae in marine water, which are vital in their primary production. Some marine invertebrates can also manufacture certain n-3 PUFAs de novo; thus, they are also considered as a crucial and fundamental source of omega-3 PUFAs. Omega-3 fatty acids produced from marine microalgae show nutraceutical and therapeutic effects. Also, the interest in delivering DHA and EPA produced by microorganisms as an alternative to fish oil sources has increased in the last couple of years.

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