# Omega-3 Polyunsaturated Fatty Acids from Algae

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# Recent Advances in Microalgal Biotechnology

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**Edited by:** Dr. Jin Liu, Dr. Zheng Sun and Dr. Henri Gerken

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# Omega-3 Polyunsaturated Fatty Acids from Algae

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## Abstract

Currently, public awareness of healthcare importance increase. Polyunsaturated fatty acid is an essential nutrition for us, such docosahexaenoic acid and eicosapentaenoic acid derivatives of omega-3. The need of omega-3 polyunsaturated fatty acid generally derived from fish oil, but fish oil has a high risk chemical contamination. Algae are single cell microorganism, which have relatively high content of omega-3. Commercial production of omega-3 polyunsaturated fatty acid is very promising for both foods and feeds, because the availability of abundant raw materials and suitable to develop in the tropics. This chapter aims to give a mini review of biology and human health benefits of using algae as a production platform for omega-3. In addition, this literature also reviews about the stimulation of omega-3 production and biotechnological approach in algae with respect to both challenges and opportunities.

## Introduction

Omega-3 is unsaturated essential fatty acids which cannot be synthesized by human due to lack of desaturase enzyme acts to insert double bonds at ω3 position [1]. There are three types of omega-3 fatty acids involved in human physiology are α-linolenic acid (ALA), Eicosapentaenoic Acid (EPA), And Docosahexaenoic Acid (DHA).ALA Is The Shortest Chain Of N-3 and mainly found in vegetables oil and nuts; other two important derivatives EPA and DHA found in fish and many other microorganisms, such as microalgae and bacteria [2-5]. Even, ALA can be converted into EPA and DHA in the body, but the conversion is very limited and inefficient [3], therefore omega-3 must be provided in the form of dietary supplement. These long chains Polyunsaturated Fatty Acid (PUFA) in algae have profound benefits and functions in dietetics and therapeutic uses [6-8]. They are believed to have positive effects for the treatment of hypertension, premenstrual tension, hypertonia, cancer, hyperlipidemia and number of other cases [7-11]. In addition, ALA is the precursors of prostaglandins (E2 and F2) which possess potent vasodilators, antiflammatory and antiaggrgatory properties as well as may be useful to correct defects occur in metabolism of essential fatty acid and imbalance of EPA [12]. EPA plays an important role in mammals as an agent to prevent blood platelet aggregation [13-15] whereas DHA is significant for the reception and transmission

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of impulses between brain cells [16]. Furthermore, dietary omega-3 (T3) PUFAs especially EPAand DHA are plays a major role in modulating the biosynthesis of eicosanoids and in controlling the levels blood lipids and lipoproteins [17]. Currently, some government agencies and nutritional organizations recommended daily intake level of DHA and EPA ranging from 0.2-0.3 g/day for the general population [18-19] up to 1.0- 4.0 g/day for patient with coronary heart disease [18].These important properties make omega-3 (T3) PUFAs valuable chemicals with extensive applications in dietary supplements, pet nutrition, beveragesand pharmaceutical industries. Worldwidemarket analysis in consumption of PUFAs estimated at 123.8 thousand metric tons worth US$2.3 billion in 2013, is forecast to be 134.7 thousand metric tons valued at US$2.5 billion in 2014. By 2020, it is projected that demand for Omega-3 PUFAs globally would reach 241 thousand metric tons with a value of US$4.96 billion, thereby posting a volume Compound Annual Growth Rate (CAGR) of almost 10% and a value CAGR of 11.6% between 2013 and 2020 [20].

It is known that global fish stocks are in danger, so, fish production may decrease in the future. In addition to this, some fishes, especially marine fishes like salmon, sardine, tuna, anchovy, mackerel or hake, are sometimes contaminated with heavy metals as copper or mercury, and organic pollutants as PCBs or dioxins, which have a toxic effect for human health [21]. For this reason, currently algaecame forwardas the major alternative source for production of omega-3 PUFAs. There are various strains of microalgae with a wide range of components, such as proteins, carbohydrates, fats, and nucleic acids, though in varying proportions. Typically, fatty acids make up to 40 percent of microalgae mass, and some of the fatty acids are unsaturated. The proportion of PUFA differs greatly depending on the species. For example, *Crypthecodinium cohnii* microalgae species possesses only DHA and no other PUFAs; *Chaetoceros* sp., *Skeletonema costatum* and *Nannochloropsis* sp. contain predominantly EPA, whereas *Porphyridium cruentum* contains predominantly ARA.

In addition, omega-3 biosynthesis can be stimulated by sudden change of growth condition and metabolic engineering. There are several methods for extraction, purification and quantification is existing for omega-3 productions from algae through lipid extraction. Therefore, on the basis of commercial importance of omega-3 PUFAs production from algae, microalgal biotechnology have emerged with current developments in genomics, bioinformatics analyses, genetic and metabolic engineering. This article intends to provide a mini review of the recent advances in algae-based omega-3 production.

## Chemistry of Omega-3

Fatty acids are hydrocarbon-chains with a methyl group in one end of the molecule (termed omega = ω or n) and an acid group (often called carboxylic acid) in the other end. There are two major nomenclatures of fatty acids. In the most commonly used nomenclature the carbon atom next to the carboxyl group is called the alpha-carbon (α-carbon) with the consecutive carbons named β-carbon etc. The letter n is often used instead of the Greek ω to describe the methyl end. Furthermore, it is quite common to use the systematic nomenclature for fatty acids where the locations of double bonds are indicated with reference to the carboxyl group. In PUFAs the first double bond may be between the $3^{rd}$ and the $4^{th}$ carbon atom from the ω carbon, and these are called omega-3 fatty acids. Some physical and chemical property of omega-3 has been given below [22]:

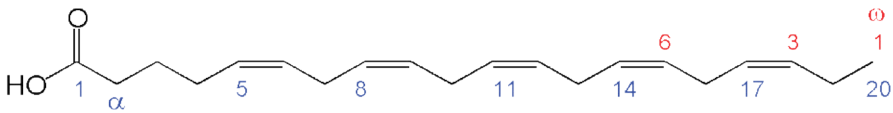
| | |
|---|---|
| **s**Molecular Weight | 909.36888 g/mol |
| Molecular Formula | $C_{60}H_{92}O_6$ |
| Hydrogen Bond Donor Count | 3 |

| | |
|---|---|
| Hydrogen Bond Acceptor Count | 6 |
| Rotatable Bond Count | 40 |
| Exact Mass | 908.689391 g/mol |
| Monoisotopic Mass | 08.689391 g/mol |
| Topological Polar Surface Area | 112 A^2 |
| Heavy Atom Count | 66 |

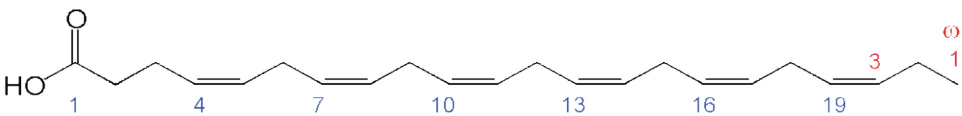
**Table 1:** Chemical and physical properties of Omega-3.

There are three most important Omega-3 fatty acids are α-linolenic acid (18:3, *n*-3; ALA), eicosapentaenoic acid (20:5, *n*-3; EPA), and docosahexaenoic acid (22:6, *n*-3; DHA) (Figure 1) [23].These three polyunsaturates have either 3, 5, or 6 double bonds in a carbon chain of 18, 20, or 22 carbon atoms, respectively. As with most naturally-produced fatty acids, all double bonds are in the *cis*-configuration, in other words, the two hydrogen atoms are on the same side of the double bond; and the double bonds are interrupted by methylene bridges (-CH 2-), so that there are two single bonds between each pair of adjacent double bonds.


a. α-linolenic acid (18:3, n-3; ALA)




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




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

**Figure 1:** Chemical structures of ALA, EPA and DHA.

The biosynthesis of omega-3 fatty acids begins with de novo synthesis of short chain fattyacids, typically oleic acid, from acetate [23]. Most organisms are capable of de novo fatty acid synthesis. Next, the oleic acid is subjected to a series of desaturation and elongation reactions to form longer chain fatty acids as can be seen in Figure 2 [23].

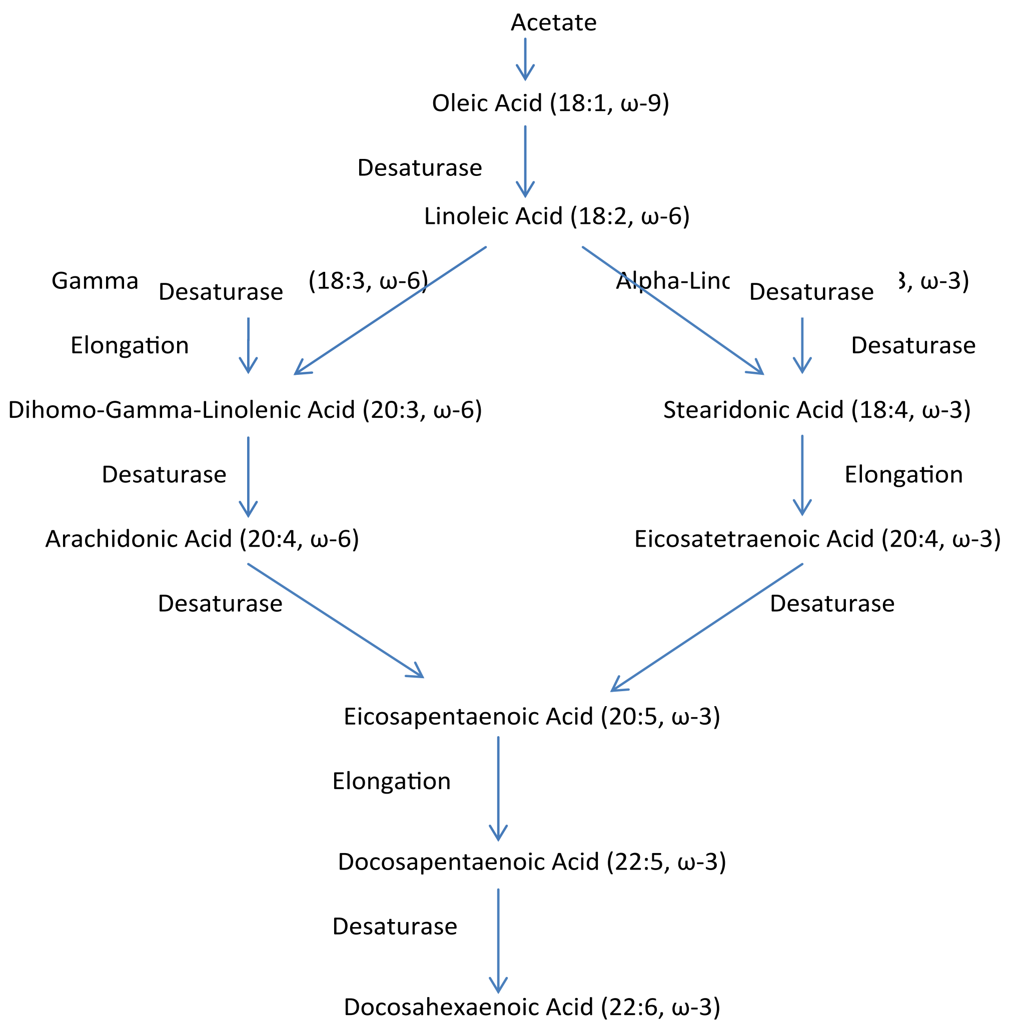



**Figure 2:** The biosynthetic pathways for omega-3 polyunsaturated fatty acids.

# Importance of Omega-3 PUFA

## Development and Growth

The combination of several months of omega-3 fatty acid deficiency and small stores of essential omega-3 fatty acids in the adiopse tissue, prepares the ground for clinical signs of omega-3 fatty acid deficiency such as scaly and haemorrhagic dermatitis, haemorrhagic folliculitis of the scalp, impaired wound healing, reduced growth in children, reduced visual acuity and neuropathy [24-31]. During foetal development the essential fatty acids are transferred from the mother via placenta to the developing foetus [32]. There are special proteins in placenta preferentially transporting DHA and AA [33-35]. Some studies suggest that supplementation with long-chain PUFA during pregnancy promotes a small increase in pregnancy duration, birth weight and a reduced risk of early preterm delivery in high risk pregnancies [36-38]. Visual functions are incompletely developed at birth, but there is a rapid development during the first year of life. DHA is a major structural lipid of retinal photoreceptor outer segment membranes. Biophysical and biochemical properties of DHA may affect photoreceptor membrane function by altering permeability, fluidity, thickness, and lipid phase properties. Tissue DHA status affects retinal cell signaling mechanisms involved in photo transduction and DHA insufficiency is associated with alterations in retinal function [39].

## Biological Effect

### Eicosanoids

Eicosanoids are important signal molecules including leukotrienes, prostaglandins, thromboxanes, prostacyclins, lipoxins and hydroxy-fatty acids. In addition, two new families of lipid mediators have been discovered, resolvins (resolution phase interaction products) and protectins, both derived from omega-3 PUFA [40]. These have potent anti-inflammatory, neuro protective and pro-resolving properties.

### Membrane Fluidity

When large amounts of very long-chain omega-3 fatty acids are ingested, there is high incorporation of EPA and DHA into membrane phospholipids, which may alter the physical characteristics of cell membranes [41]. Altered fluidity may lead to changes of membrane protein functions.

### Lipid Peroxidation

Lipid peroxidation products may act as biological signals in certain cells [42]. One of the major concerns with intake of omega-3 fatty acids has been the high degree of unsaturation and thereby the possibility of promoting peroxidation. Modified Low-Density Lipoprotein (LDL) might be endocytosed by macrophages and initiate development of atherosclerosis. Oxidatively modified LDL has been observed in atherosclerotic lesions [43], and LDL rich in oleic acid has been found to be more resistant to oxidative modification than LDL enriched with omega-6 fatty acids in rabbits [44].

### Acylation of Proteins

Acylation of proteins is important for anchoring certain proteins in membranes or folding of the proteins, and it seems to be crucial for the function of these proteins [45-46]. Although saturated fatty acids are most commonly linked to proteins, omega-3 fatty acids may also acylate proteins [47]. It has been demonstrated that PUFAs (AA and EPA) may inhibit palmitoylation and alter membrane localization of a protein kinase (Fyn) [48], whereas C14:2 may be acylated to a protein kinase (Fyn), thereby altering its raft localization and promote reduced T cell signal transduction and inflammation [49].

### Gene Interactions

Fatty acids or their derivatives (acyl-CoA or eicosanoids) may interact with nuclear receptor proteins that bind to certain regions of DNA and thereby alter transcription of these genes. The receptor protein, often interacting with another nuclear receptor, may, in combination with a fatty acid, function as a transcription factor.The omega-3 and omega-6 PUFAs are feed forward activators of Peroxisome Proliferator-Activated Receptors (PPARs), whereas these same fatty acids are feed-back inhibitors of liver X receptorS (LXRs) and Sterol regulatory element-binding proteins (SREBPs). Saturated fatty acyl coenzyme A thioesters activate HNF-4α, whereas coenzyme A thioesters of PUFAs antagonize HNF-4α action. It has also been shown that PUFAs including DHA are ligands for another transcription factor, retinoid X receptor (RXR), which is important for expression of several genes regulated by different nutrients [50-51].

### VLDL and Chylomicrons

Omega-3 fatty acids promote a striking reduction of Triacylglycerols (TAGs) in VLDL and chylomicron particles [52-54]. With daily intake of 2-4 g/day of very long-chain omega-3 fatty acids, the plasma concentration of TAGs is reduced by 20-30% [55], and the hepatic synthesis of triaglycerols is decreased, probably because omega-3 fatty acids inhibit esterification of other fatty acids in addition to being poor substrates for TAG-synthesising enzymes [56]. Very long-chain omega-3 fatty acids may also reduce TAG production by increasing fatty acid oxidation via peroxisomal β-oxidation [57].

### High Density Lipoprotein (HDL)

In most studies of human lipoprotein metabolism, gram quantities of very long-chain omega-3 fatty acids cause a small increase (1-3%) in the plasma concentration of HDL cholesterol [52, 54, 58]. Very often this increase in HDL occurs simultaneously with a fall in plasma VLDL concentration. The increased concentration of HDL cholesterol may be explained by the reduced concentration of free fatty acids in plasma [57, 59-60] causing reduced net flux of cholesteryl esters from HDL to LDL and VLDL via reduced activity of the cholesteryl ester transfer protein [61-62].

### Vascular Changes

It has been suggested that blood platelets, viscosity, coagulation and fibrinolysis might be influenced in advantageous ways by dietary intake of omega-3 fatty acids.Provision of very long-chain omega-3 fatty acids to healthy volunteers as well as to subjects with increased risk of cardiovascular disease, reduced the generation of platelet activating factor (PAF; important for platelet aggregation) [63-64], which may be of importance for suppression of rolling and adherence of monocytes on activated endothelial cells [65]. Supplementation with omega-3 fatty acids may reduce blood pressure, in particular among hypertensives [66-68]. From a meta-analysis of clinically controlled trials it has been estimated that systolic and diastolic blood pressures are decreased by 0.66 and 0.35 mmHg, respectively, per gram of very long-chain omega-3 fatty acids supplied through the diet [67]. It is also possible that EPA and DHA might improve endothelial function [69] via eicosanoids or working as ligands for transcription factors.

## Diseases

### Cardiovascular Diseases

Cardiovascular diseases are mainly caused by atherosclerosis, and give rise to development of myocardial infarction, cerebral infarction, cognitive decline, and gangrene and loss of function in the extremities.It has been shown that dietary factors such as saturated and trans fatty acids, cholesterol, some coffee lipids and sodium, in addition to lack of omega-3 fatty acids may promote development of atherosclerosis [70-74].

### Atherosclerosis

The effects of very long-chain omega-3 fatty acids on some parameters of importance for the development of atherosclerosis are outlined. Some of these effects of omega-3 fatty acids have been demonstrated in controlled clinical trials, whereas other findings refer to studies in animals or *in vitro* [42, 55, 75-77]. Among the positive effects of omega-3 fatty acids are reduced activity of blood platelets via thromboxanes (TXA2), reduced Platelet Activating Factor (PAF), reduced PDGF, reduced chemotactic effect via Leukotrienes (LTB4) And Relaxation Of Smooth Muscle Cells Via EDRF Which Promotes Increased Blood Flow and reduced blood pressure.

### Arrhythmias

In cells and animal studies it has been shown that incorporation of marine omega-3 fatty acids reduces the risk of arrhythmias, probably due to inhibition of the fast voltage-dependent sodium-channel [78-79]. Arrhythmias causing sudden cardiac death often arise from ischemia-induced electrical instability in the heart muscle. Ischemia may promote depolarization of cardiac membranes by reducing the activity of sodium/potassium ATPase, which enhances interstitial potassium concentration, making the resting membrane potential more positive. This may make myocytes more likely to depolarize due to small stimuli and thereby initiate an arrhythmia [80].

### Cancer

In spite of the fact that animal fat is associated with many of the carcinogenic effects of dietary fat [81], there is some scientific evidence that omega-3 fatty acids may protect against development of certain types of cancers [82-83]. Narayanan et al., [84] observed altered expression of genes important for apoptosis and regulators of colon cancer cell proliferation, explaining how DHA might protect against colonic cancer development. Another explanation for the inhibitory effect of omega-3 fatty acids on colorectal cancer may be due to cytotoxic peroxidation products generated during lipid peroxidation of EPA and cyclooxygenase (COX) activity [85]. Feeding experiments with rodents showed that omega-3 fatty acids decreased colon carcinogenesis at both the initiation and promotion stages [86-88]. A large number of studies in animals and in cultured cells demonstrate that omega-3 fatty acids may inhibit initiation, growth and metastasis of malignant cells [65].

### Inflammatory Diseases

A significant number of reports indicate that supplementation with a few grams daily of very long-chain omega-3 fatty acids provide health benefits in relation to some inflammatory diseases [76,79]. The mechanism behind these effects may be related to altered eicosanoid formation or any of the other mechanisms described. For the eicosanoids, it has been shown that LTB5 derived from EPA may replace some of the more potent inflammatory agent LTB4 derived from AA. Omega-3 fatty acids may inhibit the adhesion of monocytes and endothelial cells possibly due to generation of platelet activating factor [65].

### Rhematoid Arthritis / Joints

Several studies have shown that dietary supplementation with very long-chain omega-3 fatty acids (>3 g/day) reduces the clinical symptoms of rheumatoid arthritis as evaluated by morning stiffness and number of swollen joints, in meta-analyses [86-90].

### Asthma and Allergy

In a randomized controlled study among asthmatic children, the results suggest that dietary supplementation with long-chain omega-3 fatty acids was beneficial in a strictly controlled environment in terms of inhalant allergens and diet [91].

### Diabetes

In a meta-analysis it was concluded that intake of omega-3 fatty acids had no significant effect on glycemic control or fasting insulin [92].

## Effect on Central Nervous System

Several reports have suggested that supplementation with marine omega-3 fatty acids are important for treatment of schizophrenia, depressions or borderline personality disorder [93-97].

### Schizophrenia

Data indicate that the level of omega-3 fatty acids is low in red blood cells as well as in some cortical areas of the brain in schizophrenic patients [98-99], although the data are based on very small numbers of cases and are somewhat controversial [100].

### Alzheimer's and Parkinson Disease

The omega-3 fatty acids in fish might explain part of this beneficial effect. It is clearly possible to influence the content of DHA in the brain by dietary intake [101]. In two prospective studies it was observed that lower plasma DHA levels increased the risk of developing AD later in life [102-103]. There are too few data on Parkinson disease in relation to dietary intake of omega-3 fatty acids to conclude on the potential effects of supplementation.

## Natural Source of Omega -3 Fatty Acid

Microalgae offer a promising non-polluted resource for biotechnology and bioengineering of LC-PUFA production, as an alternative to fish oils [104-105]. Microalgae can also be grown on low to no-cost nutrients, which make them an economically viable source of omega-3 fatty acids [106]. Compared to traditional source, microalgae present a few advantages for n-3 LC-PUFA production, such as commonly occurring genes for the biosynthesis of these nutrients, simpler fatty acid profiles and higher growth rates.

Various auto- and heterotrophic marine species from different classes produce EPA and DHA, whereas AA is generally found in scarce amounts [107-108]. According to recent reviews of total lipid extracts, *Bacillariophyceae* (diatoms) and *Chrysophyceae* species may be rich sources of EPA and DHA; *Cryptophyceae*, *Prasinophyceae*, *Rhodophyceae*, *Xanthophyceae*, *Glaucophyceae* and *Eustigmatophyceae* can represent interesting EPA sources, whereas DHA is found in significant amounts mostly in *Dinophyceae*, *Prymnesiophyceae*, and *Euglenophyceae* [109-110]. Moreover, some important microalge has been given in following table 2 with their fatty acid profile [111].

| | ***Chroococcus sp*** | ***Phaeodactylum tricornutum*** | ***Isochrysis galbana*** | ***Pavlova sp.*** | ***Oocystis sp.*** | ***Tetraselmis sp.*** | ***Rhodomonas baltica*** | ***Nannochloropsis oceanica*** |
|---|---|---|---|---|---|---|---|---|
| Saturated | | | | | | | | |
| C12:0 | 2.1 | – | – | – | – | – | 2.0 | 1.2 |
| C14:0 | 0.1 | 8.8 | 8.9 | 7.5 | 0.2 | 0.5 | 4.1 | 16.9 |
| C16:0 | 21.3 | 16.6 | 11.5 | 13.4 | 3.8 | 6.3 | 6.0 | 17.2 |
| C18:0 | 0.3 | 0.6 | - | 0.4 | – | 1.2 | 0.8 | 1.8 |
| C20:0 | 0.2 | – | - | – | – | – | 0.1 | – |
| C24:0 | – | 1.6 | - | – | 0.1 | – | 4.0 | – |
| Sum | 24.0 | 27.6 | 20.4 | 21.3 | 4.1 | 8.0 | 17 | 37.1 |
| Mono saturated | | | | | | | | |
| C 16:1 | 1.1 | 26.0 | 3.3 | 12.8 | 1.5 | 1.3 | 0.4 | 18.2 |
| C 18:1[c] | 0.4 | 1.8 | 13.1 | 2.9 | 3.9 | 10.7 | 3.4 | 4.1 |
| C 20:1[c] | – | – | – | - | – | 0.9 | 0.1 | 0.5 |
| C 22:1[c] | 0.1 | 0.3 | 0.6 | 0.8 | – | – | 0.1 | - |
| Sum Polyunsaturated | 1.6 | 28.1 | 17.0 | 16.5 | 5.4 | 12.9 | 4.0 | 22.8 |
| C 18:2[b] | 10.7 | 1.5 | 7.0 | 2.1 | 6.4 | 2.5 | 11.7 | 9.7 |
| C 18:3[a] | 1.0 | 0.3 | 3.8 | 1.8 | 8.1 | 6.4 | 12.0 | 0.5 |
| C 18:4[a] | – | 3.3 | 12.5 | 4.3 | 0.7 | 4.1 | 5.1 | - |
| C 20:2[b] | 0.1 | – | – | | – | – | 0.1 | 0.5 |
| C 20:4[b] | 0.1 | 2.2 | – | 0.4 | 0.5 | 0.6 | 0.2 | 3.7 |
| C 20:5[a] | – | 28.4 | 0.8 | 18.0 | 1.1 | 4.8 | 4.4 | 23.4 |
| C22:5[b] | – | 1.3 | – | | – | – | 0.2 | – |
| C22:6[a] | – | 0.2 | 15.8 | 13.2 | – | 0.2 | – | – |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sum | 11.9 | 37.2 | 39.9 | 39.8 | 16.8 | 18.6 | 33.7 | 37.8 |

**Table 2:** Fatty acid profile of some microalgae. Data are given as mg $g^{-1}$ of dry weight.

Dashes indicate FA not detected, ax-3 fatty acids, bx-6 fatty acids, cx-9 fatty acids

## Photoautotrophic Microalgae

It is generally thought that photosynthetic microalgae tend to produce higher levels of EPA than heterotrophs. *Nannochloropsis* sp., *Phaeodactylum tricornutum*, *Nitzschia laevis* and *Porphyridium cruentum* can present elevated levels of EPA in total fatty acids, although relatively low cell lipid contents tend to result in small EPA amounts in the biomass (Table 3) [112].

| Species | EPA content (%TFA)[1] | EPA content (DW)[2] |
|---|---|---|
| *Nannochloropsis* sp. | 38–39 | 2–3 |
| *Phaeodactylum tricornutum* | 31 | 5 |
| *Nitzschia laevis* | 25-33 | 3-4 |
| *Porphyridium cruentum* | 25 | 3 |
| *Odontella aurita* | 26 | - |
| *Pavlova lutheri* | 22-29 | - |
| *Cyclotella cryptica* | 17-23 | 1 |
| *Cylindrotheca* sp. | 24-25 | - |

**Table 3.** Examples of marine microalgae species characterized by EPA production.

[1]% TFA, % of total fatty acids; [2] % DW, % of biomass dry weight

## Heterotrophic Microalgae

Several marine heterotroph microalgae are considered as the most preeminent alternative industrial sources of oils rich in DHA (Table 4) [113], with approved use in human foods, especially for application in infant formulas, since they are considered to be non-pathogenic and nontoxigenic.

| Species | DHA content (% TFA) [1] | DHA content (% DW) [2] |
|---|---|---|
| *Schizochytrium mangrovei* | 31-41 | 12-21 |
| *Schizochytrium limacinum* | 25-35 | 5-15 |
| *Thraustochytrium aureum* | 32-37 | 6-7 |
| *Thraustochytrium striatum* | 37 | 2 |
| *Ulkenia* sp. | 10-23 | 5 |
| *Aurantiochytrium* sp. | 40 | 18 |
| *Crypthecodinium cohnii* | 53-57 | 5-6 |

Table 4: Examples of marine microalgae species characterized by DHA production.

[1]% TFA, % of total fatty acids; [2] % DW, % of biomass dry weight

## Stimulation of Omega-3 in Microalgae

### Environmental Stress

Omega-3 fatty acid biosynthesis can be prompted by the applying of growth environmental stresses, such as low temperature and change of salinity or UV radiation. For example, *Pavlova lutheri* increased its relative EPA content from 20.3 to 30.3 % when the culture temperature was reduced to 15°C [114]. Similarly, *Phaeodactylum tricornutum* had a higher EPA content when the temperature was shifted from 25°C to 10°C for 12 h [115]. An increase in PUFAs is expected as these fatty acids have good flow properties and would be predominately used in the cell membrane to maintain fluidity during low temperatures. Salinity may also regulate PUFA biosynthesis, although not in a consistent manner. For example, *Crythecodinium cohnii* ATCC 30556 increased its total DHA content up to 56.9% of total fatty acids when cultured in 9 g/L NaCl. Other treatments that cause the generation of reactive oxygen species and lipid peroxidation also result in higher PUFA contents. For example, *Phaeodactylum tricornutum* increased its EPA content up to 19.84% when stressed with UV light [116].

### Metabolic Engineering

Apart for external stresses, metabolic engineering is another promising approach to increase the production of omega-3 in microalgae. Synthesis of ω-3 fatty acids occurs via the elongation and desaturation of long chain fatty acids. Work has been performed to create recombinant sources of ω-3 fatty acids in a variety of systems with some success [117-118]. Canola (*Brassica napus*) seeds have been produced which overexpress the *B. napus* Δ15 desaturase, as well as the Δ6 and Δ12 desaturases from the commercially grown fungus *Mortierella alpine* to synthesize the ω-3 fatty acid Stearidonic Acid (SDA) [119]. Moreover, a promising cisgenic approach for microalgae maybe to increase EPA or DHA production by overexpressing at least some of their native elongases and desaturases. It may be necessary to use promoters inducible by external stimuli rather than constitutive promoters that may interfere with normal cell function and growth. Another, yet unexplored option may lie in the inhibition of PUFA degradation. β-oxidation of fatty acids occurs in the peroxisomes but before PUFAs can be metabolized, saturases are required to fill in the double bonds. Mutations in one or several saturases may result in less efficient β-oxidation of PUFA and a higher percentage of these fatty acids. However, at present the mechanism behind the selection and storage of fatty acids for triacylglycerol production remains unclear.

## Procedure of Omega-3 Production

Extraction of omega-3 depends on strain and physical properties of algae. A flow chart is given below for the production of omega-3 (Figure 3),

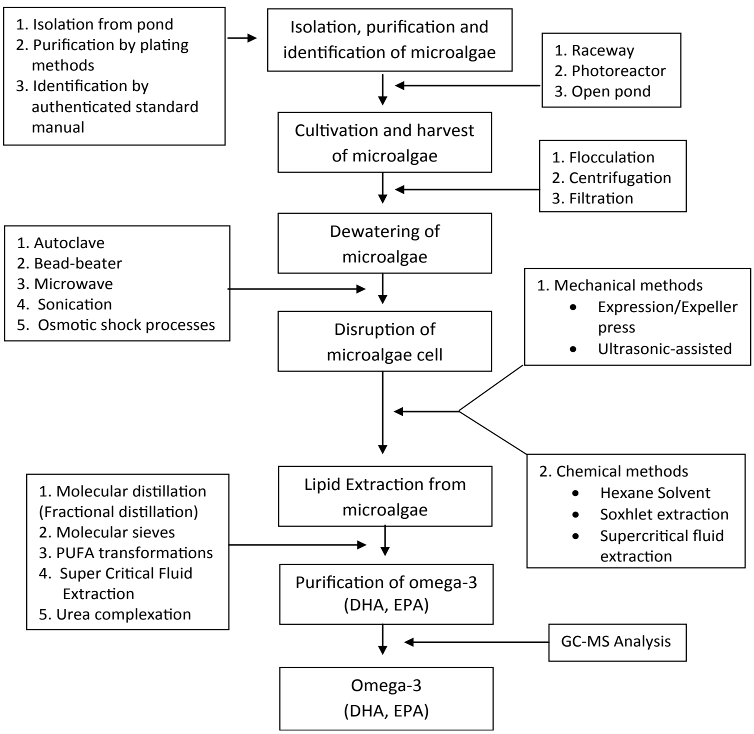



**Figure 3:** Flow chart for omega-3 production.

## Conclusion and Future Perspectives

Natural limitations favor a novel approach for the production of omega-3 fatty acids. A series of PUFAs including Eicosapentanoic Acid (EPA) and Docosahexanoic Acid (DHA) have widespread nutritional and pharmaceutical values. Microalgae cells have the potential to rapidly accumulate lipids, such as triacylglycerides that contain fatty acids important for high value fatty acids (e.g., EPA and DHA) production. However, lipid extraction methods for microalgae cells are not well established, and there is currently no standard extraction method for the determination of the fatty acid content of microalgae. Production yields are also highly dependent on microalgal cultivation conditions, biomass processing, cell disruption and strain selection in addition to solvent polarity and extraction processing.

From a commercial perspective, a techno-economic assessment is needed and should ideally be carried out for large scale extraction where costs are likely to be very different compared to the presents laboratory-based study. It is necessary to study deeper about the extraction and purification of omega-3 PUFA which is more economic with good quality and quantity along with biotechnological approach.

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