

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Bioconcentration of Marine Algae Using Lipase Enzyme

Jithu Paul Jacob

## Abstract

Marine algae rich in n-3 PUFA, being a natural and readily available resource, could be an alternative to fish oil derived n-3 PUFA; therefore, it could be of immense potentiality in nutraceutical and pharmaceutical industries. This highlights the sustainable benefits of algae and the many potential gains in creating algal bio-factories. In recent years, the use of lipase as biocatalysts had drawn considerable attention. Lipase is an enzyme that hydrolyzes lipids, the ester bonds in triglycerides, to form fatty acids and glycerol. Among the lipases assayed, the enzyme from the yeast *Candida cylindracea* is of special interest, as these are proved to be a nonspecific catalyst for many (commercially) interesting reactions such as the modification of oils and fats, reactions in organic solvents, and resolution of racemic mixtures. Hence, the enrichment of microalgae using biolipase from the source *Candida cylindracea* is of particular attention. Lipase action of *Candida cylindracea* is investigated as a function of time. It is observed that the lipases display a significant preference to saturated fatty acids; however, the resistance to release EPA and DHA was less as the hydrolysis reaction progresses.

**Keywords:** marine algae, lipase, PUFA, phospholipids, biofuels

## 1. Introduction

Poly unsaturated fatty acids (PUFAs) are unit fatty acids with a protracted chain contains 20 carbons or more, and primary covalent linkage situated on the third position carbon atom at the methyl end. PUFAs, together with EPA and DHA, proposed long before projected to bequeath health edges by rising blood pressure [1], appeasing symptoms of rheumatoid arthritis and depression, as well as attenuating the progression of Alzheimer's disease [2]. Although plant-derived  $\alpha$ -linolenic acid (ALA) is obtained from dairy products and margarines [3] and with the help of desaturase and elongase enzyme convert it to EPA and DHA in humans, where the process is inefficient (0.04–2.84%), and the conversion is restricted by high dietary intake of EPA, DHA and linoleic acid. Also, low delta-6 desaturase activity in humans may be the reason for poor conversion of ALA to EPA and DHA [4]. However, the intake of PUFA-enriched foods or marine oil supplements containing these fatty acids through diet can increase the levels of EPA and DHA. Whereas, the diet of developed countries major sources of PUFAs are fish, red meat and poultry [5] where combinations of these foods contribute high levels (>75% of the total intake derived from 29 different food groups) of DHA (fish and poultry), EPA (fish and red meat), and docosapentaenoic acid (DPA; red meat, poultry and fish).

Marine algae, like other algae, have chlorophyll a photosynthetic system and thus considering as a diverse group of photosynthetic organisms. However, they possess simple structural moiety; their reproductive structures lack sterile cells so also do not form any embryos. For broader classification generally algae are divided into eight major groups or divisions based on their difference in their photosynthetic pigments, carbohydrate reserves, and cell structures. These algal groups contain unicellular members (collectively called microalgae) and multicellular members (macroalgae or seaweeds). The application of marine algae varied greatly from human food to animal fodder, source of phycocolloids and bioactive products to even recently use for biofiltration. The economic utilization of both marine macroalgae and microalgae has been explored for some time. Since 1940, it has been used as a source of liquid fuels and single cell proteins. During 1960s, with the invention of the extraordinarily halophilic algae *Dunaliella* could be considered as the most effective natural supply of carotene, started the business utilization of microalgae gained impetus.

Nowadays, microalgae provide a wide range of use as fine chemicals, oils, and polysaccharides, as soil conditioners, waste treatment and aquaculture. As a result of their usable products, the natural resources of algae cannot meet the demand and they are overexploited in their natural habitats [6]. The cultivation of microalgae is presently one of the most productive and environmentally friendly forms of livelihood among the coastal populations. Algal culture is being investigated to be used in house vehicles as a way of air revitalisation, food production, and waste treatment.

## **2. Recovery of PUFA from microalgae**

The process has three main steps: (1) combined extraction-transesterification of fatty esters from the algal biomass; (2) a silver ion column chromatography step; and (3) a chlorophyll removal step [7]. Optimal processing conditions, the scale up of recovery, and the relative economics of producing microalgal EPA are important. The quality and stability characteristics of EPA from microalgae area unit has established. Previously, many advance process schemes introduced to purify polyunsaturated fatty acids from complex mixtures. To obtain good purity, these schemes perpetually employed as many processing operations which reduce the overall recovery and magnified costs. In several other cases, these methods had other problems which omitted it from uses. There in another study which involves complicated procedure of a two-step winterization, saponification, and urea fractionation of sardine oil successfully recovered 90% fraction of EPA and DHA, but failed to resolve those two compounds. Selective extraction of PUFA can be achieved by using aqueous solutions of silver nitrate through a water immiscible organic phase. However, this approach is questionable such that suitably it does not allow purification of a single compound such as EPA from complex mixture such as esterified oils. Similarly, PUFAs could also by selection obtained by surface assimilation of the esterified oil on aminopropyl warranted silicon oxide columns and selective extraction of saturated and oleic acid esters with solvent. The polyunsaturated fatty acid esters are then eluted with dichloromethane.

This method again had the drawback that it does not resolve highly pure EPA from the other polyunsaturated esters. For the fractionation of PUFA there is another variant of column chromatography envisions which uses aluminum oxide stationary phase and supercritical or liquid carbon dioxide as the mobile phase, but only few clear details have been published so far. Fractionation of fish oil and whole

triglyceride extracts of other marine organisms can be done directly on silica gel and Ag-impregnated silica gel, whereas, initial fractionation of whole triglycerides is not efficient for at the same time getting a high purity associate degree smart recovery of most of the Eicosapentenoic acids in an oil.

A newer novel approach used kinetic resolution to separate EPA from fish oil. Kinetic resolution is based on differences in selectivity and rates of lipase catalyzed esterification of different fatty acids in a mixture. Whereas this approach has allowed high recovery of Eicosapentenoic acids (up to 75%) from the oil, the purity of the product did not exceed 18%. When free fatty acids were used as the starting material rather than the triglycerides, EPA recovery by kinetic resolution improved to 93% but purity declined to less than 8%. Obviously, kinetic resolution as a method of purification has limited capabilities [8]. In addition, kinetic resolution using lipases under anhydrous conditions is difficult to economically implement in practice and the process is comparatively slow. Other PUFA recovery schemes, mostly useful only in the laboratory, have been reviewed elsewhere. Because of their survival in a variety of environmental conditions and widespread availability, studies on the mass culture of algae have been largely confined to freshwater species of *Chlorella* and *Scenedesmus*. According to the use of intend it is needed to screen a large number of unicellular algae from the point of view of nutritional composition, toxicity, resistance to contaminants, growth rates in mass culture, suitability etc. It is possible to mass culture in the laboratory with good results, in that cases the conditions must be good not like in primitive area where glass jars or tubes, artificial illumination, and equipment for sterilizing large volumes of seawater is not available, and in locations where the large scale culture of marine species is not possible [9].

It is important therefore to find unsophisticated methods for the production of large quantities of marine species. Other investigations into the culture of marine protist have trusted natural brine, each in open tanks and in closed controlled systems. Although impregnated natural saltwater created an honest crop of plant life, the inoculated culture was sometimes replaced rapidly by other organisms introduced with the seawater, such as motile and non-motile chlorophytes, colorless flagellates, ciliates or other zooplankters. Even underneath controlled laboratory conditions, little protist species in saltwater often experience filters and become established in culture carboys. It would be advantageous to use an artificial salt-water medium which would eliminate the immediate introduction of undesirable organisms.

Marine algae rich in n-3 PUFA being natural and readily available resource could be an alternative to fish oil derived n-3 PUFA; therefore, it could be of immense potentiality in nutraceutical and pharmaceutical industry. Lipids and protein produce during algal growth may be used as biodiesel, biomass for oil sources, and also as animal feed. This uses highlights the suitable benefits of algae and many potential gains while developing algal bio-factories. Limit factors for the potential use of marine algal oils on large scale are cost, extraction and purification methods.

In addition, extra experimentation is needed to confirm best growth conditions for enhancing macromolecule biogenesis. More over algae-derived oils are vegetarian-friendly and easy to grow on a large scale due to their small size. n-3 PUFA are typically associated with marine organisms, and algae, as the basis of the marine trophic chain that poses a very promising source of PUFA. In recent years, the use of lipase as biocatalysts had drawn considerable attention.

Lipase is associate degree catalyst that hydrolyzes lipids, the organic compound bonds in triglycerides, to create fatty acids and glycerin. Currently they account for 25% of total enzymes used in biotechnology, and this is because of the great versatility of the enzyme in catalyzing reactions of hydrolysis and synthesis,



interesterification and transesterification. Among the lipases, the enzyme from the yeast *Candida cylindracea* is of particular interest, since these are proved to be a nonspecific catalyst for many (commercially) appealing reactions such as the modification of oils and fats, resolution of racemic mixtures and reactions in organic solvents [10]. Hence, the enrichment of microalgae using biolipase from the source *Candida cylindracea* is of particular attention. The partial hydrolysis of the sardine oil by *Candida cylindracea* lipase indicates a strong discrimination by the lipase against DHA, so the DHA present in triglycerides does not get hydrolyzed, in effect get concentrated [11].

On the other hand, this lipase has only moderate discrimination against EPA, so the concentration percentage of EPA is comparatively lower than that of DHA with a moderate enrichment. Thus, the partial hydrolysis values of the sardine oil by *Candida cylindracea* lipase indicate higher specificity of lipase towards DHA than towards EPA. Lipase action of *Candida cylindracea* is investigated as a function of time. It is observed that the lipases display a significant preference to saturated fatty acids, however, the resistance to release EPA and DHA was less as the hydrolysis reaction progresses. It has been reported that because n 3 PUFA is located in the second position of triglyceride, hydrolysis of sardine oil with 1,3 specific lipase should produce PUFA rich 2-monoglycerides and 1,2 diacyl glycerides [12, 13]. The presence of cis carbon-carbon double-bonds in the fatty acids result in bending of the chains. Therefore, the terminal methyl group of the fatty acids lies so close with the ester bond that can cause a steric hindrance effect on lipases. Due to the presence of five and six double bonds there is a high bending effect of EPA and DHA, enhancing the steric hindrance effect; consequently lipases cannot reach the ester-linkage between these fatty acids and glycerol. However, saturated and mono-unsaturated fatty acids of triglycerides do not possess any barriers to lipases so they can be easily hydrolyzed. Therefore, fatty acid selectivity of the lipases for EPA and DHA allows their separation and concentration from other components left behind portion of marine oils. In addition to it, the lipases have been frequently used for the discrimination between EPA and DHA in concentrates containing both of these fatty acids, thus providing the possibility of producing omega3-PUFA concentrates with dominance of either EPA or DHA.

The enzyme lipase was first commercially successfully introduced by Novo Nordisk in 1988 under the trade name “Lipolase”. It was actually originated from the fungus *Humicola lanuginosa*. Again, in 1995 two bacterial lipases were introduced—“Lumafast” and “Lipomax” from *Pseudomonas mendocina* and *Pseudomonas alcaligenes*, respectively both produced by Genencor International. Currently, industrial enzymes are manufactured by three major suppliers, they are Novozymes, Denmark, Genencor International Inc., US and DSM NV, Netherlands. Lipases are marketed by various brand names like Lipopan, Lipozyme, Novozyme, Patalase, Greasex, Lipolase and Lipoprime. Lipases of microbial origin have gained considerable attention in the field of biotechnology and a large number of microbial strains have been used for the enzyme production.

The production of extracellular lipase by *Candida cylindracea* in a batch bioreactor is influenced by aeration, substrate type and concentration. Both olive oil and oleic acid when used as the carbon sources gave almost identical activity while the production of extracellular lipase was growth associated. For optimum lipase production it required the enrichment of air flow by pure oxygen by maintaining the oxygen concentration at the recommended value. The optimal growth conditions for lipase production by *Candida cylindracea* is influenced by agitation speeds and aeration in a fermentor [14]. Maximum lipolytic activity was observed when the microorganisms were at the beginning of the stationary growth phase. For the production of lipase submerged cultivations using yeast has been found to be the

most suitable process. Meanwhile, these processes are influenced by a variety of parameters and also their interactions. Due to the complex morphology of the cells, industrial processes involving submerged fermentation require greater attention.

The fermentation runs with 200 rpm yielded higher protein activity compared with the fermentations runs at 400 rpm. Higher rpm speed light-emitting diode to the formation of cells to aggregates with vacuolation. The vacuoles formed clumps which lowered the enzyme production. When the agitator speed was 200 rpm the vacuoles will be separated, resulting in increased enzyme production [15]. Aeration and dissolved oxygen also affected the morphology of the cell. Yeasts are considered as important sources for lipase production. There are problems such as changes in morphology of cells during agitation and while enzyme production the combined effect of operational parameters have negative effect on enzyme preparation in fermentation steps.

For the enrichment or bio concentration of DHA and EPA in marine oils lipases are used. These can be applied in free fatty acids (FFA) or in simple esters of marine oil fatty acids also. One of the major advantages is that the lipases can be operated under mild conditions, so that products such as EPA and DHA are preferable since they are prone to oxidation. The choice of lipase and raw material used were depended upon the structure of the desired lipid and ratio of EPA to DHA in the final product. Specificity of the lipase towards the fatty acid should also be considered. The location of the fatty acids is in triacylglycerols (TG), and then the regiospecificity and TG specificity also have an effect on the enrichment. Thus, the positional distribution of the fatty acids on the acylglycerol molecule structure may have an effect on the ability of the lipase to enrich DHA and/or EPA in either the substrate or product. Different strategies are employed using lipases for the concentration of EPA and/or DHA of marine origin. Lipases from *Candida rugosa* (formerly *Candida cylindracea*) and *Rhizomucor miehei* discriminate against DHA than EPA. But those from porcine pancreas, *Chromobacterium viscosum*, *Pseudomonas* sp., *Pseudomonas cepacia* and *Pseudomonas fluorescens* do vice versa, i.e.: discriminate against EPA than DHA. Lipase from *Rhizomucor miehei* can be applied for the enrichment of DHA in FFA from fish oil by alcoholysis of the oil with butanol or glycerol. By ethanolysis of fish oil this lipase succeeded in separating DHA into the acylglycerol fraction and EPA into the ethyl ester fraction [16]. Lipase from *Candida rugosa* also got similar application in catalyzing the enrichment of DHA in the acylglycerol fraction by the hydrolysis of fish oil. Both DHA and EPA enrichment has been successful in the acylglycerol fraction obtained by ethanolysis or hydrolysis of fish oil catalyzed by the lipase from *Pseudomonas* sp., *Pseudomonas fluorescens* and *Geotrichum candidum* [12, 13]. The lipase from *Rhizopus delemar* has been used to catalyze the esterification between FFA and lauryl alcohol to concentrate DHA in the FFA from fish oil. To concentrate both DHA and EPA in FFA another approach is to esterify FFA from marine origin with glycerol catalyzed by the lipase from *Pseudomonas* sp., *Pseudomonas fluorescens*, *Thermomyces lanuginosus* (formerly *Humicola lanuginosa*) or *Rhizopus oryzae*.

### 3. Analysis of enriched fatty acid in marine algae

The reagents commonly used for acid-catalyzed transesterification are methanolic, hydrochloric and sulfuric acid, and boron trifluoride in methanol. All of them are suitable for lipid transesterification and also free-fatty-acid methylation. However, at ambient temperature neither acid-catalyzed nor boron-fluoride-catalyzed reactions proceed; in both cases the reaction requires heating. Among the mentioned reagents, boron trifluoride-methanol reagent (12–14% w/v) is the

most often used for transesterification of all types of lipids and it is being the best and very useful reagent for lipid esterification. Under the conditions recommended (heating at 100°C), transesterification is complete within 2 min for free fatty acids, within 10 min for phosphoglycerides, within 30 min for triglycerides and within 90 min for sphingomyelin. For biological samples boron fluoride-methanol reagent is used for transesterification of lipids following the procedure given below: into a screw-capped tube (Teflon cap liner) a small aliquot of the lipid extract (dissolved in chloroform) is added; to it add 0.5–1 ml boron fluoride-methanol reagent (140 g/l, containing an required amount of BHT as antioxidant); the tube is then closed and heated at 90°C for 2 h. This methodology proved to be administered complete transesterification of all lipids; the addition of BHT can prevent rancidity of PUFA.

After the transesterification reaction completed, FAMES are extracted by adding n-hexane and water twice to the proportion of sample. This is the most simple and effective procedure. Even though, this method is most popular, boron tri-fluoride-methanol has few disadvantages. Unless refrigerated, the reagent has only a limited shelf life [17]. The use of recent or too focused solutions might lead to the assembly of artifacts or loss of PUFAs.

If the sample contains plasmalogens, aldehydes area unit liberated by the chemical agent and area unit regenerate into dimethyl acetals (DMAs), that area unit nearly not possible to break free the major carboxylic acid methyl esters including methyl palmitate. There is wide usage of anhydrous methanolic hydrochloric acid and methanolic sulfuric acid for lipid esterification varying under different conditions, in particular, different reaction temperatures, different acid concentrations, and different reaction times. The methanolic acid boron fluoride-methanol works as methylating free fatty acids very rapidly so can be used to transesterify all the lipids which are typically present in the biological samples. With a concentration of 5% methanolic hydrochloric acid complete transesterification can be carried out by heating the sample in the reagent for about 2 h under refluxion. This reaction can also be carried out at 50°C overnight. In the same way a solution of 1–2% (v/v) concentrated sulfuric acid in methanol can be used for the transesterification of lipid samples. This method using methanolic, hydrochloric and sulfuric acids also have the disadvantage like boron trifluoride-methanol that DMAs are formed during transesterification from plasmalogens. Acetyl chloride and aluminum chloride are the other reagents used for transesterification. Both these reagents shown complete transesterification in the samples without prior extraction of the lipid, whereas, aluminum chloride has the disadvantage that it does not esterify free fatty acids.

#### **4. PUFA as phospholipids fractions**

Phospholipids are major constituents of cell membranes and play essential roles in biochemistry and physiology of the cell functions. Phospholipids in fish and marine species are highly enriched with the long-chain n-3 type polyunsaturated fatty acids. About 40–50% content of EPA and DHA is not uncommon in some phospholipid classes in fish. The role of n-3 polyunsaturated fatty acids in phospholipid moiety is in adjusting the membrane integrity and functions presumably at lower temperature, and also to the membrane fluidity and mobility as a result of their higher unsaturation. In the case of fish among the phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are by far the most abundant in the flesh, especially PC make up to 50–60% of the total phospholipid content [18]. The composition of individual phospholipid classes is remarkably similar among fish species as is the characteristic fatty acid composition of each



class. Lecithins present in plant and vegetable origin are popularly using as health supplements. The vegetable oil is highly enriched with n-6 fatty acids, which so as in the case of the n-3 fatty acid fish oils. On the other hand, purified fish lecithins, which are highly enriched with n-3 polyunsaturated fatty acids phospholipids, are not available on the market at all. This is because tedious extraction procedure is required for obtaining lecithin from fish oil unlike plant or vegetable lecithins. Here, certain attempts for the preparation of such phospholipids, highly enriched with EPA and DHA, from the more readily available plant or animal lecithins is explained. Pure phosphatidylcholine can be obtained from egg yolk after purification by preparative HPLC and was treated under the acidolysis reaction using the *Mucor miehei* lipase. There is observed reaction as anticipated in which the rate of the reaction involving the phospholipids that possess the zwitterionic head group. This is much lower as compared to the natural triacylglycerol substrates. Therefore, large quantities of lipase were required, which will resulted in high extent of hydrolysis side reaction. The optimal reaction conditions is offered in a mixture of phospholipids of approximately 40% desired for phosphatidylcholine and lyso-phosphatidylcholine (LPC) whereas, 20% of glycerol phosphatidylcholine (GPC). In LPC only one of the acyl moiety will be hydrolyzed and in glycerol phosphatidylcholine both acyl groups will get hydrolyzed. When pure EPA was used, both the PC and the LPC fractions were highly enriched with EPA, particularly the LPC fraction, 58 and 69%, respectively.

## 5. Application of PUFA

PUFA had many helpful effects for human health so it considered as unit important elements in human nutrition. The intake of PUFA in diet, together with n-6 fatty acids, is understood to modulate the inflammatory processes among different cell functions. Although many of the species exhibited high amounts of SFA, some *Phaeophyta* and *Rhodophyta* species show higher concentrations of PUFA, and PUFA/SFA ratios higher than 1 (*H. scoparia*, 1.46; *T. atomaria*, 1.33; *C. spongiosus*, 1.77; *Peyssonnelia* sp., 1.33). Whereas, the lowest ratios were discovered in algae from the phylum Chlorophyta (0.27–0.68) [19]. It seems that this phylum incorporates a lower potential, examination to the opposite two phyla studied, as a nutritional source of PUFA for human consumption. However, not all PUFA are associated with the promotion of health benefits. For example, in the inflammation process, eicosanoids derived from n-6 PUFA are generally considered as pro-inflammatory or as promoters of other cell harmful effects, whereas n-3 PUFA derivatives are considered less inflammatory or even anti-inflammatory [20].

Since the synthesis pathway of those fatty acids depends on identical enzymes for n-3 and n-6 PUFA, the health promoting effects area unit keen about the n-6/n-3 magnitude relation of PUFA obtained through diet.

The World Health Organization (WHO) recommends a  $\sum n-6 / \sum n-3$  magnitude relation not up to 10. Almost all algae can be considered as a good source of dietary PUFA, since they showed ratios ranging between 0.29 and 6.73 [21]. The exception was *Chaetomorpha* sp., during which the  $\sum n-6 / \sum n-3$  magnitude relation was the best from all the studied species (31.25) and in *D. spiralis* during which no n-3 fatty acids were detected. Besides associate degree applicable nutritionary profile, these macroalgae can also be exploited for pharmaceutical purposes.

Many of the PUFA thought-about powerful molecules against many diseases and area unit already employed in totally different medical specialty applications. For example, several reports suggest that n-3 fatty acids, mainly EPA and DHA, may have a significant potential in the treatment of autoimmune and inflammatory



diseases. Rhodophyta was the phylum with the highest percentage of n-3 fatty acids (16–27% of total FAME), followed by Phaeophyta (0–15%), in which significant amounts of n-3 were also present. Aside from *Ulva* sp. that had 18 of n-3 FAME, Chlorophyta macroalgae conferred very cheap values of n-3 fatty acids (1–9%). Conversely, the detected n-6 fatty acids were lower in Rhodophytes (8–15%), thanks to the low concentration of linoleic acid, except for *Peyssonnelia* sp., where n-6 concentration was approximately 28% of total FAME. Phaeophytes showed the highest contents of n-6 fatty acids (23–44%), whereas chlorophytes presented mid-range values (6–27%).

Considering absolutely the concentrations of PUFA within the varied species tested, *Ulva* sp., *T. atomaria*, *C. spongiosus*, *Peyssonnelia* sp. and *B. secundiflora* possess the best contents of n-3 PUFA, 1.07, 1.38, 1.19, 1.06 and 1.42 mg/g, severally. Apart from genus *Ulva* sp., during which ALA dominated, the n-3 profile of the remaining strains was basically composed of independent agency. Nevertheless, *Peyssonnelia* sp. exhibited a relatively high content of DHA, 0.22 mg/g of dry biomass, coupled with an EPA concentration of 0.84 mg/g. A variety of potential applications area unit delineated for independent agency and DHA, which hold significant potential for pharmaceutical purposes, namely cancer treatment, asthma, psoriasis, rheumatoid arthritis, antibiotic, inflammatory bowel disease, depression, allergies, cardiovascular diseases, among others [22].

More recently, PUFA verified to own a robust potential in drug delivery; additionally to the delineated toxicity of a number of PUFA, PUFA enable a more efficient penetration of specific molecules through the cell membranes of tumor cells, due to their unique lipophilic characteristics. In fact, several studies show that tumor cells display faster PUFA intake than normal cells, as demonstrated for the conjugated taxoid DHA-paclitaxel. The nutritional and pharmaceutical edges of PUFA, however, contrast with the increasing difficulty in finding sustainable sources of n-3 VLCPUFA, which traditionally were obtained from fish and fish oil [23].

Declining fish stocks caused by decades of overfishing makes ever more urgent to find non-traditional alternatives for the western world. As VLCPUFA are usually absent from terrestrial higher plants, traditional crops can also be excluded as viable sources of these FA. Though this deficiency can be overcome by applying genetic engineering, transgenic foods are not always well accepted by the general public. Therefore, n-3 VLCPUFA are typically associated with marine organisms, and algae, as the basis of the marine trophic chain, come out as a very promising source of VLCPUFA. In fact, large scale farming of marine algae has been accomplished successfully for hundreds of years. Approximately, 220 protocist species area unit presently cultivated and harvested everywhere the globe for various functions. Though principally used as food for human consumption, particularly in Asia, macroalgae are also the primary source of hydrocolloids such as agar, carrageenan and alginate, which have numerous industrial applications, such as gelling, stabilizing or binding agents. The next step may somewhat be the property exploitation of marine macroalgae as different sources of VLCPUFA, not solely in Asia, however conjointly within the western world.

IntechOpen

IntechOpen

### **Author details**

Jithu Paul Jacob  
St. Albert's College (Autonomous), Ernakulam, Kerala, India

\*Address all correspondence to: [jithupaul007@gmail.com](mailto:jithupaul007@gmail.com)

### **IntechOpen**

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

## References

- [1] Fortin PR, Lew RA, Liang MH, Wright EA, Beckett LA, Chalmers TC, et al. Validation of a meta-analysis: The effects of fish oil in rheumatoid arthritis. *Journal of Clinical Epidemiology*. 1995;**48**:1379-1390
- [2] Martins JG. EPA but not DHA appears to be responsible for the efficacy of omega-3 long chain polyunsaturated fatty acid supplementation in depression: Evidence from a meta-analysis of randomized controlled trials. *Journal of the American College of Nutrition*. 2009;**28**:525-542
- [3] Astorg P, Arnault N, Czernichow S, Noisette N, Galan P, Hercberg S. Dietary intakes and food sources of n- and n-3 PUFA in French adult men and women. *Lipids*. 2004;**39**:527-535
- [4] Ballesteros A, Bemabe M, Cruzado C, Martin-Lomas M, Otero C. Regioselective deacylation of 1,6-anhydro-beta-D-galacto-pyranose derivatives catalyzed by soluble and immobilized lipases. *Tetrahedron*. 1989;**45**:7077-7082
- [5] Howe P, Meyer B, Record S, Baghurst K. Dietary intake of long-chain  $\omega$ -3 polyunsaturated fatty acids: Contribution of meat sources. *Nutrition*. 2006;**22**:47-53
- [6] Li CY, Chen SJ, Cheng CY. Production of *Acinetobacter radioresistens* lipase with repeated fed-batch culture. *Biochemical Engineering Journal*. 2005;**25**:195-199
- [7] Deleuze H, Langrand G, Millet H, Baratti I, Buono G, Triantaphylides C. Lipase catalyzed reactions in organic media: Competition and applications. *Biochimica et Biophysica Acta*. 1987;**911**:117-120
- [8] Monot F, Borzeis F, Bardin M, Vandecasteele JP. Enzymatic esterification in organic media: Role of water and organic solvent in kinetics and yield of butyl butyrate synthesis. *Applied Microbiology and Biotechnology*. 1991;**35**:759-765
- [9] Noor IM, Hasan M, Ramachandran KB. Effect of carbon and nitrogen sources on the production of lipase by *Candida cylindracea* 2031 in batch fermentation. In: *Proceedings of the 1st International Conference on Natural Resources Engineering and Technology*; 2006. pp. 158-166
- [10] Felony G, Armas JC, Mendoza JCD. Production of extracellular lipase from *Aspergillus niger* by solid-state fermentation. *Food Technology and Biotechnology*. 2006;**44**:235-240
- [11] Fadiloglu S, Erkmen E. Effects of carbon and nitrogen sources on lipase production by *Candida rugosa*. *Turkish Journal of Engineering and Environmental Sciences*. 2001;**26**:249-254
- [12] Otero C, Pastor E, Ballesteros A. Synthesis of monobutyl-glycerol by transesterification with soluble and immobilized lipases. *Applied Biochemistry and Biotechnology*. 1990;**26**:35-44
- [13] Ciafardini G, Zullo BA, Iride A. Lipase production by yeasts from extra virgin olive oil. *Journal of Food Microbiology*. 2006;**23**:60-67
- [14] Carta G, Gainer JL, Benton AH. Enzymatic synthesis of esters using an immobilized lipase. *Biotechnology and Bioengineering*. 1991;**37**:1004-1009
- [15] Benzonana G, Esposito S. On the positional and chain specificities of *Candida cylindracea* lipase. *Biochimica et Biophysica Acta*. 1971;**231**:15-22
- [16] Otero C, Pastor E, Fernandez VM, Ballesteros A. Influence of the support

on the reaction course of tributyrine hydrolysis catalyzed by soluble and immobilized lipases. Applied Biochemistry and Biotechnology. 1990;**23**:237-247

lipids in normal, hypertensive and hyperlipidemic subjects. Prostaglandins, Leukotrienes, and Medicine. 1986;**24**:173-193

[17] Sokolovska I, Albasi C, Bales V. Production of extracellular lipase by *Candida cylindracea* CBS 6330. Bioprocess Engineering. 1998;**19**:179-186

[18] Burdge GC, Finnegan YE, Minihane AM, Williams CM, Wootton SA. Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [ $^{13}\text{C}$ ]  $\alpha$ -linolenic acid to longer-chain fatty acids and partitioning towards  $\beta$ -oxidation in older men. The British Journal of Nutrition. 2003;**90**:311-321

[19] Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Wilson RS, et al. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Archives of Neurology. 2003;**60**:940-946

[20] Block RC, Harris WS, Pottala JV. Determinants of blood cell omega-3 fatty acid content. The Open Biomarkers Journal. 2008;**1**:1-6

[21] O'Sullivan TA, Ambrosini G, Beilin LJ, Mori TA, Oddy WH. Dietary intake and food sources of fatty acids in Australian adolescents. Nutrition. 2011;**27**:153-159

[22] Geleijnse JM, Giltay EJ, Grobbee DE, Donders ART, Kok FJ. Blood pressure response to fish oil supplementation: Metaregression analysis of randomized trials. Journal of Hypertension. 2002;**20**:1493-1499

[23] Singer P, Berger I, Wirth M, Gödicke W, Jaeger W, Voigt S. Slow desaturation and elongation of linoleic and  $\alpha$ -linolenic acids as a rationale of eicosapentaenoic acid-rich diet to lower blood pressure and serum