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ALGAL OIL: A NOVEL SOURCE OF OMEGA-3 FATTY ACIDS FOR HUMAN NUTRITION

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Abstract

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) a polyunsaturated fatty acid (PUFA) that belongs to the omega-3 fatty acids group. In recent years, omega-3 fatty acids rich oil has attracted much attention because of its recognized beneficial effect on human health. At present, fish oil is the major source of omega-3 fatty acids, but omega-3 fatty acids rich oils can be produced by microalgae with additional commercial benefits. Consumption of omega-3 rich fish oil has been steadily increasing for decades due to their health benefits. Microalgal oil might be the most promising alternative to fish oil, since they are the primary producers of omega-3 fatty acids. Recent advances regarding production of omega-3 fatty acid rich oil from microalgae have been reviewed with an emphasis on the scientific data. At first, description of the omega-3 fatty acids, biosynthesis pathways and their role in the human health is presented. Microalgae are the initial source of omega-3 fatty acids. Microalgae species intensively used in omega-3 fatty acids rich algal oil production and their culturing conditions were reviewed in this paper. The algal oil extraction and refining process are also presented. We present here a review of the most recent advances regarding the production of omega-3 fatty acid rich algal oil from the marine origin microalgae.

***Key words**: Omega-3 fatty acids, docosahexaenoic acid, eicosapentaenoic acid, microalgae, algal oil.*

INTRODUCTION

Polyunsaturated fatty acids (PUFA), specifically docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have been linked to several health benefits (Armenta & Valentine, 2013; Covington, 2004). Several studies have suggested there is positive health effects consumption of DHA and EPA, such as lowering the risk of vision loss due to eye macular degeneration, reduction of blood vessel stiffness, and relief of anxiety and inflammation (Ho, van Leeuwen, Witteman, van Duijn, Uitterlinden, Hofman, et al., 2011; Kiecolt-Glaser, Belury, Andridge, Malarkey, & Glaser, 2011; Pase, Grima, & Sarris, 2011). DHA consumption has been associated with reducing risk of colds in babies from moms that took this dietary supplement (Imhoff-Kunsch, Stein, Martorell, Parra-Cabrera, Romieu, & Ramakrishnan, 2011). All the latter, plus numerous studies that suggest there are health effects for the heart and brain, and many other benefits for treating several illnesses and diseases including asthma, rheumatoid arthritis, schizophrenia, depression, multiple sclerosis, migraine headaches, etc (Gray, 2011). Even low DHA levels have been associated to suicide risk (Armenta & Valentine, 2013; Lewis, Hibbeln, Johnson, Lin, Hyun, & Loewke, 2011).

The major sources of EPA and DHA in food and dietary supplements were found in fatty fish, fish products, marine oils, and certain algae oils (Andız & Ünlüsayın 2015). At present, the major resource of omega-3 fatty acids for human nutrition is marine fish oils. However, their reserves are dwindling, and supplies via wild fish fisheries are limited. Moreover, omega-3 fatty acid content of oil from wild-caught fish varies with species, location, water depth and temperature, seasonal climatic conditions, and availability and type of primary food chain (Khozin-Goldberg, Iskandarov, & Cohen, 2011). A variety of fish species such as herring, mackerel, sardine and salmon are regarded as good sources of omega-3 fatty acids. Due to the many shortcomings of fish-derived oil including undesirable taste and odour, diminishing supplies, objections by vegetarians, its chemical processing methods, and the presence of contaminants such as mercury, dioxins and polychlorinated biphenyls (Certik & Shimizu, 1999; Hooper, Thompson, Harrison, Summerbell, Ness, Moore, et al., 2006), research has been diverted towards the exploitation of other marine species for the development of a suitable and sustainable alternatives (Gupta, Barrow, & Puri, 2012). Microalgae offer a promising non-polluted resource for biotechnology and bioengineering of omega-3 fatty acid oil production, as an alternative to fish oil. Compared to terrestrial crop plants, microalgae present a few advantages as omega-3 fatty acids sources, such as commonly occurring genes for the biosynthesis of these nutrients, simpler acid profiles and higher growth rates (D. A. Martins, Custodio, Barreira, Pereira, Ben-Hamadou, Varela, et al., 2013). Most of oil producing microalgae studied within the past decade have been eukaryotes; and have been found worldwide, both along coastlines and in the open ocean. Using microalgae to produce omega-3 fatty acid rich algal oil is still a relatively new field, and research on this area has been growing significantly within the last few years (Armenta & Valentine, 2013). Studied microalgae include *Nannochloropsis oculata (Pal, Khozin-Goldberg, Cohen, & Boussiba, 2011) Pavlova lutheri*, *Odontella aurita* (Guihéneuf, Fouqueray, Mimouni, Ulmann, Jacquette, & Tremblin, 2010), *Schizochytrium* sp. (I. Fedorova-Dahms, P. A. Marone, M. Bauter, & A. S. Ryan, 2011), *Crypthecodinium cohnii* (Mendes, Reis, Vasconcelos, Guerra, & da Silva, 2009), *Ulkenia* sp. (Quilodrán, Hinzpeter, Hormazabal, Quiroz, & Shene, 2010). This review focus on recent advances made in biotechnological production of omega-3 fatty acids rich oil from microalgae alternative to fish oil.

Omega-3 fatty acids

Polyunsaturated fatty acids (PUFAs) constitute a large group of fatty acids containing long chain carbonic molecules that include ω-3 and ω-6 fatty acids. Omega 'ω' is the position of the first double bond when counted from the methyl end and the number '3' refers to the number of carbon atoms at that position from the methyl end. The molecular structure of the fatty acids consists of an even number of carbon atoms (4 to 24) with diverse saturations (0 to 6 double bonds) (Gupta, Barrow, & Puri, 2012). Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are two members of the ω-3 family (Figure 1).

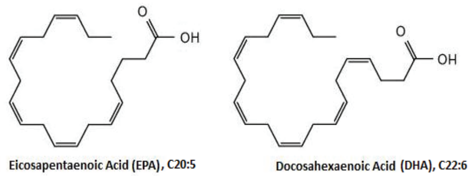

Figure 1. Structure of common omega-3 fatty acids: DHA and EPA.

Biosynthesis pathways of EPA and DHA are shown in Figure 2. The prefixes 'docosa' and 'eicosa' are of Greek descent, meaning the 22 and 20 C atoms present in DHA and EPA, which contain 6 and 5 cis-double bonds respectively (Lozac'h, 1986). PUFAs rich in omega-6 fatty acids originate from terrestrial plants are consumed in higher abundance than those from fish, so that plant origin ω-6 fatty acids are consumed in higher quantities than ω-3 fatty acids (Newton, 1998). This over consumption of ω-6 relative to ω-3 oil has been linked to increased risk of cancer, diabetes, cardiovascular and neurodegenerative diseases (A. Simopoulos, 2006). To restore a balance, consumption of ω-3 fatty acids should be increased compared to ω-6 fatty acids. ω-6 to ω-3 PUFA ratios of between 5:1 and 3:1 have been suggested as optimum for human consumption (A. P. Simopoulos, 2008).

Omega-3 fatty acids rich microalgae

In the marine food system, polyunsaturated fatty acids are primarily formed by phytoplankton and transferred on to herbivorous zooplankton, hence affecting food quality for organism at higher trophic levels. Various photoautotrophic and heterotrophic marine species from different classes produce EPA and DHA omega-3 fatty acids.

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* (Lang, Hodac, Friedl, & Feussner, 2011; D. A. Martins, et al., 2013).
These organisms offer a promising vegetative and non-polluted resource for biotechnology and bioengineering of omega-3 fatty acids rich algal oil production as an alternative to fish oil.
Currently, the production of omega-3 fatty acids by marine microorganisms is the subject of intensive research and increasing commercial attention (L. Sijtsma & M. E. de Swaaf, 2004). Several photosynthetic and heterotrophic marine microalgae are considered as a good source of omega-3 fatty acids (EPA and DHA) for the commercial production of algal oil (Table 1).

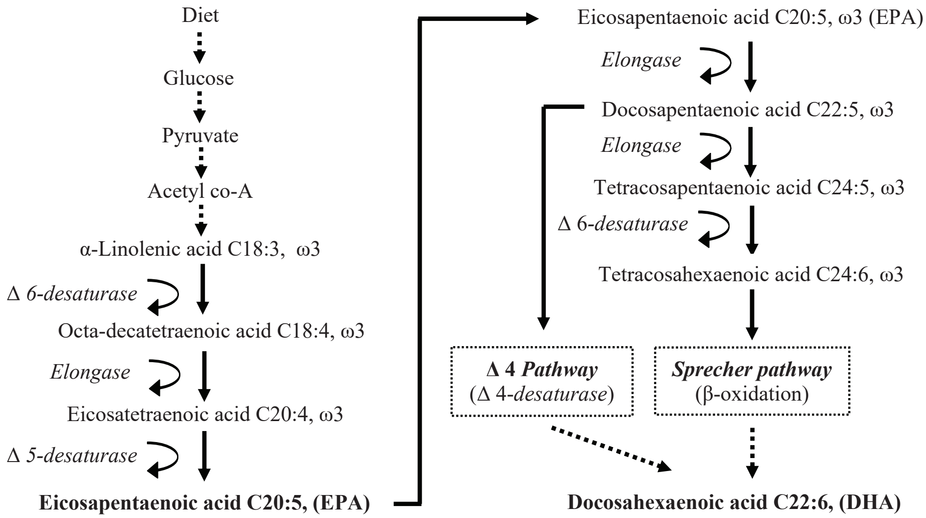

Figure 2. Biosynthesis pathways of EPA and DHA ω-3 fatty acids.

Photoautotrophic microalgae

It is generally thought that photosynthetic microalgae tend to produce higher levels of EPA than heterotrophs. *Nannochloropsis* sp. Hibberd, *Phaeodactylum* Bohlin, *Nitzschia* Hassall and *Porphyridium* Nägeli 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 (D. A. Martins, et al., 2013) (Table 1). *Pavlova lutheri* and *Nannochloropsis occulata* are marine microalgae commonly used in aquaculture, is a well-known source of n-3 LC-PUFA, such as EPA and DHA under specific conditions such as during exponential growth using low light (Guihéneuf, Mimouni, Tremblin, & Ulmann, 2015; Winwood, 2013).

Heterotrophic microalgae

Currently, the most common micro-algae used for the production of DHA rich algal oil are from the marine members of the families *Thraustochytriaceae* and *Crypthecodiniaceae*.
The Thraustochytrids include the genera *Schizochytrium* sp. and *Ulkenia* sp., whereas *Crypthecodinium* is a genus of the family Crypthecodiniaceae (Table 1). Thraustochytrids, which includes the genera Schizochytrium and Thraustochytrium, are among the most promising microorganisms for producing omega-3 fatty acids, with reported oil contents of >50 % (dry basis) and more than 30 % DHA within the total oil produced (Burja, Radianingtyas, Windust, & Barrow, 2006). Among the heterotrophic marine microalgae, *Crypthecodinium cohnii* was identified as a prolific producer of DHA. The *C. cohnii* is extraordinary in that it produces no other PUFAs other than DHA in its cell lipid in any significant amount (Mendes, Reis, Vasconcelos, Guerra, & da Silva, 2009; Van Pelt, Huang, Tschanz, & Brenna, 1999).

Table 1. Microalgae species intensively used in omega-3 fatty acids rich algal oil production

Microalgae	Culture condition	Omega-3 fatty acids	References
Schizochytrium sp.	Heterotrophic	24-45 % DHA, 10 % EPA	(I. Fedorova-Dahms, P. Marone, M. Bauter, & A. Ryan, 2011; Hammond, Mayhew, Naylor, Ruecker, Mast, & Sander, 2001)
Ulkenia sp.	Heterotrophic	35-40 % DHA	(Dulce Alves Martins, Custódio, Barreira, Pereira, Ben-Hamadou, Varela, et al., 2013)
Crypthecodinium cohnii	Heterotrophic	25-60 % DHA	(De Swaaf, Sijtsma, & Pronk, 2003; L. Sijtsma & M. De Swaaf, 2004)
Thraustochytrium striatum	Heterotrophic	37 % DHA, 23 EPA	(Fan, Chen, Jones, & Vrijmoed, 2001)
Aurantiochytrium sp.	Heterotrophic	40 % DHA	(Hong, Rairakhwada, Seo, Park, Hur, Kim, et al., 2011)
Pavlova lutheri	Phototrophic	12-18 % DHA, 22-28 % EPA	(Guihéneuf, Mimouni, Ulmann, & Tremblin, 2009)
Nannochloropsis sp.	Phototrophic	38-39% EPA	(Chaturvedi & Fujita, 2006)
Phaeodactylum tricornutum	Phototrophic	40-57 % EPA	(Fernández, Pérez, Sevilla, Camacho, & Grima, 2000)
Nitzschia laevis	Phototrophic	25-33 % EPA	(Xiao-Hong, Song-Yao, WANG, & Mei-Fang, 2007)
Porphyridium cruentum	Phototrophic	25 % EPA	(Durmaz, Monteiro, Bandarra, Gökpinar, & Işik, 2007)

Extraction and purification of algal oil

After growth and harvesting of microbial biomass, oil must be gently extracted. Nonetheless, there is no universal method that will yield the best oil recovery for algae. In some algae cells, cell walls are particularly thick and cell disruption method must be employed for recovery process. Extraction of algal oil for the production of omega 3 fatty acids is difficult process because as soon as the algal cell walls are ruptured, these PUFAs are exposed to potential oxidation. Once these highly unsaturated fatty acids have reacted with oxidized radicals, an unstoppable chain reaction begins which leads to the production of rancid, highly odorous oil which is unsuitable for human consumption. Hence, so far as possible, all sources of materials that can initiate the oxidation process should be eliminated during the extraction and storage period (Winwood, 2013). Crude vegetable oils, including algal oils, require refining to improve color, clarity, odour and remove any particulate material and chemical contaminants. There are wide ranges of impurities in the crude oil that can be removed by the refining process, including: free fatty acids, phosphatides (i.e. lecithin), pigments (i.e. carotenoids, chlorophyll), trace metals, sterols (i.e. cholesterol), waxes, mono acyl and diacyl glycerides, waxes, oxidation products and trace contaminants. Therefore, oil refining needs to be performed to remove non-triglyceride, colorants, smelly and toxic compounds in the production of edible oil (Rubio-Rodríguez et al., 2010). The conventional oil refining in industry is usually made by chemical methods, which include several steps as degumming, to separate phospholipids; neutralization or de-acidification, to clear free fatty acids and decrease oil acidity; bleaching to absorb pigments or contaminants and deodorization to remove smelly compounds. Supercritical fluid technology, together with membrane and enzymatic processes, is one of the most recent technologies proposed as alternative to oil refining with chemical products or high temperatures (Čmolík & Pokorný, 2000).

CONCLUSIONS

Scientists have a great opportunity to discover and exploit many as yet unidentified marine microbes capable of producing higher levels of omega-3 fatty acids and other valuable products. One of the main challenges is developing optimum culture conditions for rapidly growing marine microbes that produce high levels of omega-3 fatty acids, as compared to the limited number of commercially useful species presently available in the international depository. The application of metabolic engineering techniques to improve the wild-type strain is in an early stage of development. The bioengineering can further improve yields of omega-3 fatty acids from algae. Consumers are aware of the importance of an adequate provision of these nutrients and several properties of microalgal oils are particularly appealing, such as their sustainability, high purity and quality, "vegetarian" origin, and improved organoleptic qualities when compared to fish oils. Although genetically modified crops will likely serve as omega-3 sources in the future, microalgae oils have a great potential to present purer profiles, which are highly advantageous during processing and may address differentiated purposes in the market.

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