Microalgae: A Promising, Yet Challenging, Source of Sustainable Omega-3 PUFAs

Mahdi Hassan Zadeh

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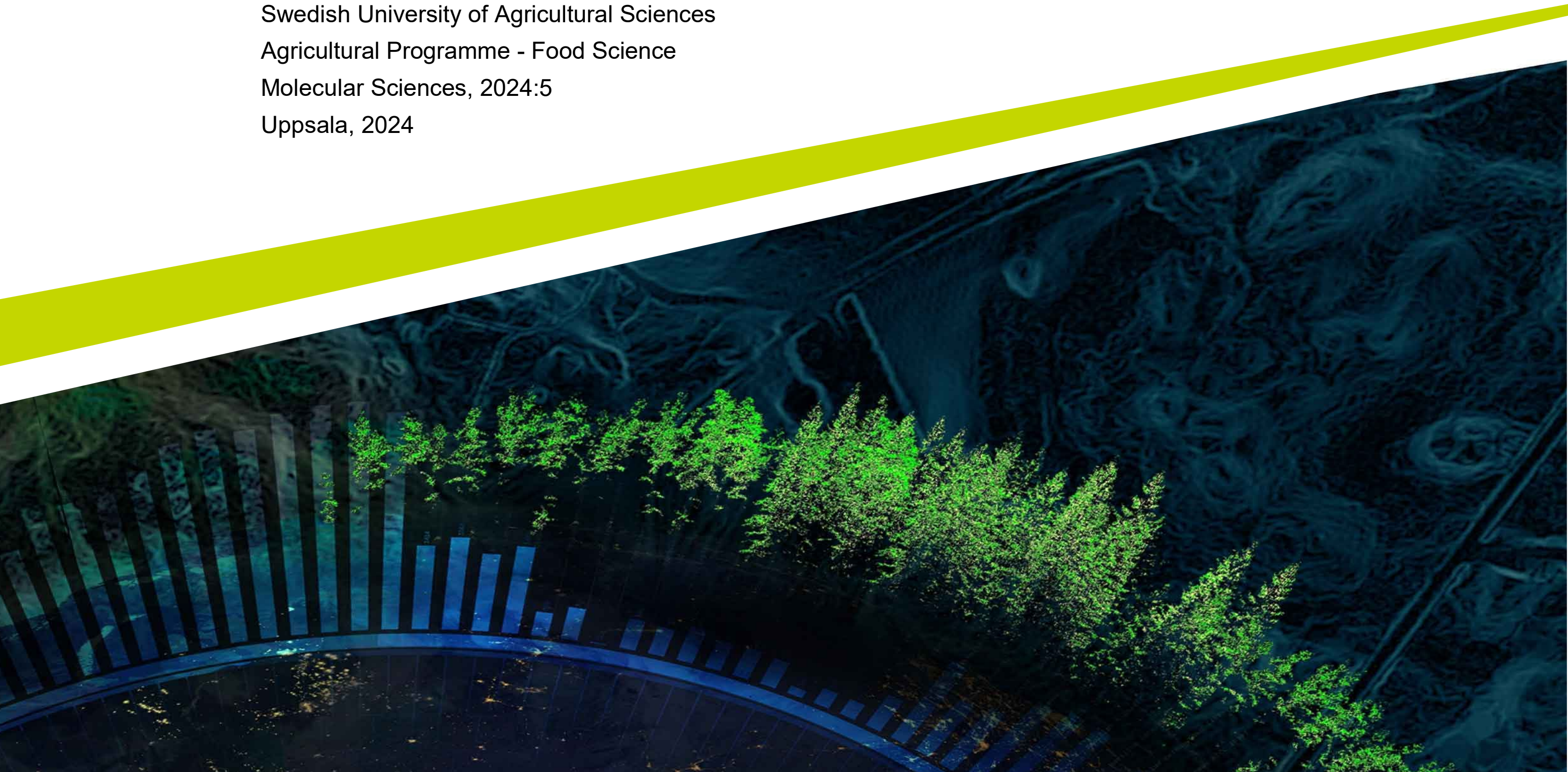

Microalgae: A Promising, Yet Challenging, Source of Sustainable Omega-3 PUFAs

Mahdi Hassa Zadeh

Supervisor:	**Jana Pickova, SLU, Department of Molecular Science**
Examiner:	Saeid Karkehabadi, SLU, Department of Molecular Science

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Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Science
Department of Molecular Science

Abstract

Humans need omega-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Despite being involved in many functions such as cell signaling, anti-inflammatory reactions, and more, the body can only synthesize them in a small amount, which makes them semi-essential in this manner. Furthermore, the sustainability of these traditional sources, like fish oil raises questions as the increasing world population leads to a greater demand. Microscopic water creatures known as microalgae provide potential as a sustainable substitution, as they are naturally able to produce DHA and EPA, which can be used for an efficient supply of these fatty acids.

The kingdom of microalgae is incredibly diverse. Certain species, such as *Crypthecodinium* and *Nannochloropsis*, are acknowledged leaders in the production and accumulation of PUFAs. It is important to study this biodiversity and choose strains with high PUFA content in order to maximize yield.

Some efforts are needed to obtain microalgal oil. There are a range of culture techniques, such as phototrophic cultivation (using sunlight), heterotrophic cultivation (using organic materials), and mixotrophic cultivation (using both). The processes of harvesting and drying microalgae are also important, as well as methods of cell disruption for effective lipid extraction and purification to produce an oil with a higher ratio of PUFA.

The production of PUFAs in microalgae is influenced by the surrounding environment. Factors such as the availability of vital nutrients (nitrogen and phosphorus), environmental pH and salinity of the environment, the delicate balance of temperature, light intensity, and wavelength, and the effect of CO_2 concentration are briefly examined in this thesis. Microalgae have significant potential, and there are opportunities for development, such as manipulating cultivation factors to increase PUFA content.

Production costs are one of the challenges in scaling up microalgal oil manufacturing. Nevertheless, the researchers show a positive picture while acknowledging these difficulties. These challenges may be solved by further studies and advancements in culture techniques, opening the door to producing a cheaper and more sustainable PUFA from microalgae.

This study intends to create a better understanding of production and affecting factors for the production of PUFAs (particularly EPA and DHA) by microalgae. This may also help to give an outlook of microalgae as a new potential source of PUFAs, which can play a major role in enhancing human health in the future.

Keywords: microalgae, PUFA, LC-PUFA, EPA, DHA, production steps, abiotic factors

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Abbreviations

PUFA	Polyunsaturated fatty acid
ω-6	Omega 6
ω-3	Omega 3
ALA	α-linolenic acid
EPA	Eicosapentaenoic acid
DPA	Docosapentaenoic acid
DHA	Docosahexaenoic acid
ARA	Arachidonic acid
LA	Linoleic acid
LC-PUFA	Long-chain polyunsaturated fatty acid
CO_2	Carbon dioxide
SE	Solvent extraction
SFE	Supercritical fluid extraction
FFA	Free fatty acid
MUFA	Monounsaturated fatty acid
SFA	Saturated fatty acid
LTC	Low-temperature crystallization
HPLC	High-performance liquid chromatography
SFC	Supercritical fluid chromatography

1. Introduction

Polyunsaturated fatty acids (PUFAs) are fatty acids with two or more double bonds within the carbon chain. Generally, in PUFA, a methyl group is at one end of the molecule (termed omega, "ω" or "n") and a carboxyl group is at the other end. According to the position of the first double bond from the methyl group terminal, PUFAs are classified into two known families: Omega-3 (ω-3) and omega-6 (ω-6) (Wiktorowska-Owczarek et al. 2015; Mariamenatu & Abdu 2021). For instance, α-linolenic acid (ALA; 18.3 ω-3), eicosapentaenoic acid (EPA 20:5 ω-3), and docosapentaenoic acid (DPA; 22:5 ω-3), and docosahexaenoic acid (DHA; 22:6 ω-3) are important ω-3, whereas linoleic acid (LA; 18:2 ω-6) and arachidonic acid (ARA; 20:4 ω-6) some important ω-6 fatty acids (Saini & Keum 2018). The amphipathic (hydrophilic head and hydrophobic tail) and other properties of PUFAs make them contribute to numerous biological functions such as maintaining cell membrane fluidity, anti-inflammatory reactions, cell signaling, the regulation of blood pressure, nervous system, and hematic clotting (Wiktorowska-Owczarek et al. 2015; Kapoor et al. 2021). An inadequate intake of ω-3 PUFAs can be related to numerous health issues such as cardiovascular disease, diabetes, cancer, Alzheimer's, and depression in addition to fetal, visual, and brain underdevelopment (Swanson et al. 2012; Shahidi & Ambigaipalan 2018).

Long-chain polyunsaturated fatty acids (LC-PUFA) such as EPA and DHA are semi-essential ω-3 fatty acids, humans can either biosynthesize them from the essential PUFA ALA (at a low level) or accumulate directly from food. Among the traditional sources of ω-3 LC-PUFAs, aquatic origin such as fish and shellfish, cover almost 90% of the intake (Demets & Foubert 2021). However, there are many concerns around the sustainability of fish oil including overfishing as well as increasing consumption due to rising populations, technological developments, and awareness of ω-3-related health benefits, and risk of the presence of contaminants (e.g., organic pollutants and heavy metals) (Kothri et al. 2020). Wild fish accumulate EPA and DHA through the consumption of microalgae and phytoplankton present in different fish and other animals whereas the terrestrial alternatives (e.g., rapeseed oil) often used as fish feed in aquaculture lack these LC-PUFAs (Demets & Foubert 2021; Sivakumar et al. 2022).

Some studies mentioned that the ratio of ω-6/ ω-3 in the Western diet is 20-50/1 compared to the required value of 1-4/1 (DiNicolantonio & O'Keefe 2020; Mariamenatu & Abdu 2021). The excessive intake of ω-6 over ω-3 and under-consumption of DHA and EPA can increase the pro-inflammatory agents from ω-6s and decrease the anti-inflammatory agents from ω-3s, which are associated with inflammation, cancer, and other chronic diseases. Thus, new and sustainable sources of PUFAs (especially ω-3) seem to be increasingly in demand (Adarme-Vega et al. 2014; Mariamenatu & Abdu 2021; Wei et al. 2021).

Some of the new and reliable alternatives to produce omega-3 LC-PUFAs are through oleaginous microorganisms such as microalgae, oil from krill and other aquatic lower food chain organisms, as well as transgenic terrestrial crop plants (Harwood 2019; Kothri et al. 2020; Zhou et al. 2023).

1.1 Aim

The thesis in this study aims to collect and summarize some of the existing knowledge regarding the production and enhancement factors of microalgae lipids as novel and relatively promising alternative sources of PUFAs. The main focus is on ω-3 fatty acids, particularly DHA, and EPA in addition to some ω-6 fatty acids such as LA, and ARA. Furthermore, the challenges in production and development as well as the advantages of algal oil are briefly discussed.

1.2 Method

For the literature study, I used online databases, including ScienceDirect, PubMed, Google Scholar, Scopus, and Primo, to collect the presented information. The keyword combination during the searches includes algal oil, microalgal, PUFAs, omega 3, omega 6, EPA, DHA, fish oil, sustainability, abiotic factors, health benefits, and essential fatty acids.

2. Microalgae as an LC-PUFA source

The terminology of microalgae refers to all microscopic algae, which are unicellular species that can live individually or in chains and groups (Winwood 2013a). They can be found in the ocean as well as in freshwater. The estimated total species number is between 200,000 and 800,000 and their size can be up to a couple of hundred micrometers. Phytoplankton are photoautotrophic microalgae species that can synthesize organic materials from carbon dioxide (CO_2) in the presence of light. They belong to different phyla, including the Bacillariophyta (e.g., *Chaetoceros, Phaeodactylum, Skeletonema, Thalassiosira*), Chlorophyta (e.g., *Tetraselmis*), Cryptophyta (e.g., *Cryptomonas, Rhodomonas*), Haptophyta (e.g., *Isochrysis, Pavlova*), Heterokontophyta (e.g., *Nannochloropsis*) and Rhodophyta (e.g., *Porphyridium*). Microalgae can also be cultivated heterotrophically, during which the organic molecules will be taken up as primary sources of nutrition in a way similar to fermentation. Some known heterotrophs microalgae are *Chlorella, Nitzschia, Cyclotella, Tetraselmis, Schizochytriumand, and Crypthecodinium* species (Ryckebosch et al. 2012).

Microalgae produce a range of compounds for adaptation and survival in different environmental conditions. For instance, the stored fatty acids depend on the microbial growth stage and function as an energy source during unfavorable conditions or cell division. Microalgae are the primary producers of ω-3 LC-PUFAs in aquatic food systems. EPA and DHA are produced as acyl parts of structural lipids because of their high flow properties, which increase membrane fluidity under high temperatures and hyper-pressure conditions. Additionally, the antioxidative properties of these LC-PUFA are also established in marine microorganisms. Thus, EPA and DHA are commonly synthesized and accumulated in microalgae in response to growth-limiting stresses such as low light and temperature, salinity, and nutrient depletion. Thus, the composition of algal oil is a product of both culture conditions and the species (Adarme-Vega et al. 2012, 2014; Santin et al. 2021).

2.1 Some known PUFA-rich microalgae

The fatty acid content of different microalgae ranges from 20-70% of the total microalgae, but the most commonly found are those with a length chain of 14, 16, and 18 carbons (Barros de Medeiros et al. 2022). The PUFAs are also biosynthesized in high quantities in very diverse amounts. For instance, LA (ω-6) is 25% and 23% (of total lipid content) in respectively, *chlorella vulgaris* and *Porphyridium purpureum*. Moreover, *P. purpureum* also accumulates ARA in a high amount (39%), which indicates the presence of a high amount of ω-6 PUFA. On the other hand, the ALA ratio in some algae species seems to be notable, including, *Dunaliella salina* (36%), *Ectocarpus siliculosus* (30%), and *C. vulgaris* (30%) (Harwood 2019). Additionally, some species have the potential to store EPA, such as *Nannochloropis* (up to 61% lipids), *Pophyridium* (up to 18% lipids), and *Phaeodactylum* (up to 41% lipids), respectively up to 49%, 40%, and 36% of total lipids (Demets & Foubert 2021; Ren et al. 2022). Furthermore, *Crypthecodinium* species (up to 50% lipids) can possess a DHA ratio of up to 79% of total lipids under certain conditions, like very low temperatures (Moniz et al. 2022). Additionally, DHA is also the major PUFA produced by *Schizochytrium* spp. at levels often exceeding 40% of total fatty acids where the total fatty acid is on average 62% (Chi et al. 2022).

2.2 Production of Microalgal Oil

The production of microalgae includes several steps, namely, cultivation, harvesting and drying, lipid extraction, and purification.

2.2.1 Cultivation

Phototrophic (autotrophic) cultivation is the most common method used to grow microalgae on a large scale for various applications. EPA-producing species tend to be more phototrophic, where the source of energy comes from light and CO_2. This method is used consistently due to the low cost of scaling up, lower risk of unwanted environmental effects, and reduced probability of contamination. Open-pond in addition to closed photobioreactor systems are the two most popular techniques in photoautotrophic (Dubey et al. 2024). Despite some disadvantages in a closed photobioreactor, namely, the higher operational cost, inadequate light absorption, and challenges of scaling up, advantages include reduced contamination risk, no diffusion of CO_2 to the atmosphere, more control over cultivation conditions (e.g., light, temperature, and nutrients), and flexibility in design of cultivating facilities, make closed photobioreactors considerably beneficial over the open-pond (Astiasarán & Ansorena 2009; Russo et al. 2021).

In heterotrophic cultivation, microalgae employ organic carbon sources such as glucose, acetate, glycerol, etc. Thus, the implementation of no need for light and CO_2 allows it to be a cheaper alternative to phototrophic cultivation. The similarity to conventional fermentation can result in a higher cell density using different techniques, including fed-batch, perfusion culture, etc. However, the number of heterotropic species used for EPA production is very limited (e.g., *Thraustochytrium*, *Chlorella,* and *Scenedesmus*). Nevertheless, commercially produced DHA by *Schyzochytrium* spp. has already been available since the 1990s (Chi et al. 2022). Light-induced metabolites such as pigments are not synthesized at adequate levels, which is an additional drawback of heterotrophic cultivation (Castillo et al. 2021; Sivakumar et al. 2022). In addition, the use of a carbon source requires sterilization to hinder the possibility of any contamination with other heterotrophic microorganisms such as bacteria, yeast, and fungi (Barta et al. 2021).

Depending on the availability of organic or inorganic carbon sources in the presence or absence of light, some microalgae can switch their mechanism of nourishment between autotrophic and heterotrophic, which are called mixotrophs. The advantages of this technique are, namely, higher growth rate, more effective nighttime biomass production, and less photo-oxidative damage. An example is certain EPA-producing species such as *Nannochloropsis spp.*, whose biomass concentration increased by 40% in mixotrophic cultivation (Dubey et al. 2024).

2.2.2 Harvesting and Drying

The harvesting involves the separation of the slurry (microalgae biomass) from the media to achieve a higher cell density, which tends to contribute up to 30% of the total cost (Liu et al. 2022). It is considered as the initial step that makes further processing possible. The harvesting technique is chosen based on the size, shape, and the toughness of cell wall. The separation and harvesting can be done either mechanically or chemically.

The common mechanical methods of harvesting are, namely, centrifugation, filtration, sedimentation, and floatation, whereas the chemical-based method includes flocculation (Christenson & Sims 2011; Vasistha et al. 2021). Centrifugation, which results in an effective separation, is being widely used on a commercial scale (Wang et al. 2019). Filtration is employed as a step before centrifugation by using membranes (e.g., polyvinylidene fluoride) to reduce energy consumption as well as more effectively retain microalgae. Flocculation involves the agglomeration of particles into larger units, which increases the rate of sedimentation and is usually used before centrifugation. The methodology is based on the neutralization of the surface charge (more negative) by positive ions (e.g., iron chloride, aluminum sulfate, and cation polymers) to overcome the repulsion between cells and thus promote cell aggregation (Guedes et al. 2011; Nitsos et al. 2020; Sivakumar et al. 2022).

The next step is drying of the harvested matter to extract the oil as well as extend the storage time. The common drying techniques include spray drying, freeze drying, heat drying, and vacuum drying, where each technology has its own merits and demerits (Mata et al. 2010). In spray drying, the microalgae get dispersed into very small droplets, and then hot air gets applied upon them for a very short time, which results in a short evaporation time. Despite being costly, spray drying is more compatible with commercial-scale production since it demands less energy compared to the other methods (Ren et al. 2022; Zhang et al. 2022).

2.2.3 Pretreatment and Extraction

The extraction of algae lipids is challenging due to the cell wall of microalgae, which protects the oil content. The cell wall consists of several layers, including cellulose and pectin, which are very rigid and resistant to get through (Jothibasu et al. 2022). As a result, it makes lipid recovery extremely expensive. The cell wall should be destroyed to collect the inside oil, which can be done mechanically and/or non-mechanically. The method of choice depends on the biology and the characteristics of the cell wall of microalgae. The mechanical cell disruptions include high-pressure homogenization, bead milling, ultrasonication treatment, steam explosions, and pulsed electric fields. Although these methods have a high product recovery on a commercial scale, they are considered to be energy-demanding (Lee et al. 2017; Alhattab et al. 2019).

Non-mechanical cell wall disruptions include chemical treatments (e.g., acid, surfactant, nanoparticle, and osmotic shock), and biological treatments (e.g., enzymatic and algicidal). Cell membranes interact with chemical reagents or enzymes, resulting in the cell wall's breakdown. Despite being less expensive than mechanical approaches, the risk of toxicity and thermolabile compound degradability is still concerning (Nunes et al. 2023).

After the cell disruption, extraction can be more convenient. The methods include mechanical (e.g., oil expeller, ultrasonic, ultrasonic-assisted, and microwave-assisted) and chemical (e.g., solvent extraction and supercritical fluid or CO_2 extraction). The mechanical approaches are more employed in the biodiesel production sector, whereas the chemical methods are the ones used in oil manufacturing (Mubarak et al. 2015).

In solvent extraction methods (SE), the principal is to dissolve non-polar compounds such as lipids and fatty acids (often) into hydrophobic solvents. Organic solvents like hexane, chloroform, acetone, and methanol are used to effectively extract the oil from algae biomass. The key factor in SE is the penetration of solvent into the biomass to maximize extraction. The oil composition and recovery vary when different solvent combinations are used for different microalgae. (Mercer & Armenta 2011). Studies have shown that a combination of chloroform and methanol is the most efficient solvent for the extraction of PUFAs such as DHA, EPA, and ARA (Otero et al. 2017). Some elements, such as a higher temperature and a pressure above the boiling point, can result in better extraction due to increasing the capacity of the solvent to solubilize solutes, especially after drying the biomass (Richter et al. 1996). Solvent extraction possesses some drawbacks, such as the possibility of the presence of a residual solvent, inorganic salt, and heavy metals, in addition to higher costs (solvent removal) and lower recoveries (Mercer & Armenta 2011).

Supercritical fluid extraction (SFE), also known as green extraction technology, depends on the critical pressure and temperature of the used solvent (Zinnai et al. 2016). This condition allows the solvent to have a viscosity similar to that of gas or liquid form, which increases the mass transfer between the extractants and the supercritical fluid (Esquivel-Hernández et al. 2017). CO_2 is widely used due to its moderate critical pressure and low temperature. In some cases, by optimizing the extraction conditions, the PUFA yield increased. In particular, the pressure and density of CO_2 increased (22 MPa and at 50ºC) which resulted in higher PUFAs in the triglycerides extracted from *B. braunii* (Mubarak et al. 2015). SFE presents some advantages, like being safe (not having toxins and impurities), fast, and having a lower lipid oxidation rate (anaerobic condition). On the contrary, the SFE demands a special instrument to induce and handle the high pressure, which is considered a major challenge due to the high initial cost (Li et al. 2019).

2.2.4 Purification

The extracted lipids contain several impurities, such as free fatty acids (FFA), monounsaturated fatty acids (MUFA), saturated fatty acids (SFA), odor, taste, and pigments, which need to be removed to obtain concentrated PUFA oil (Li et al. 2019; Chen et al. 2023; Karrar et al. 2024). The methods employed to purify PUFAs include winterization, urea inclusion, low-temperature crystallization (LTC), molecular distillation, chromatographic separation, and more. (Li et al. 2019).

During urea inclusion, a mixture of fatty acids and urea gets crystallized after saponification (removal of sterol and vitamins) during which SFAs and MUFAs form inclusion complexes with urea while PUFAs remain in the solution. Although urea complexation demands a lot of raw materials, simplicity, low cost, and low energy consumption make it a very commonly used method for purifying PUFAs from extracted oil (Li et al. 2019; Kermanshahi pour et al. 2020). Additionally, urea inclusion also hinders the oxidation of ω-3 PUFAs. In a study (Gu et al. 2009), by using urea gradient cooling, 91% of LA was collected.

LTC has some advantages, such as low cost, simplicity, and convenient operation. However, the requirement for cooling equipment, the usage of large amounts of organic solvent, and the low rate of separation and recovery indicate some limitations and challenges of this method (Li et al. 2019).

Another method to purify PUFAs is chromatography. Some techniques like high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC), among others, are applied to obtain EPA and DHA from extracted oil. SFC is based on the supercritical fluid phase, in which the purification is based on the degree of solubility between the mobile phase and methylated fatty acids. The combination of liquid and gas chromatography makes the separation occur (Karrar et al. 2024). It should also be mentioned that the DHA and EPA are more effectively separated in SFC than in HPLC (Perrut & Clavier 2003).

FFAs are often eliminated from the mixture by chemical methods such as alkalization. Decolorization is performed by absorbent clay or activated carbon to remove color pigments as well as oxidized agents. To improve clarity, the oil gets de-waxed through winterization due to the high melting points of waxes. Moreover, high-pressure steam under a vacuum condition is applied to the oil to get rid of the remaining unwanted residues that can deviate taste, odor, and color (Winwood 2013b). There is no perfect purification method to obtain highly purified oil, which indicates that a combination of various methods needs to be employed to optimize the purity considering other factors such as cost and energy consumption (Ren et al. 2022).

3. Factors and Techniques Affecting PUFA Content

Several physicochemical factors, as well as environmental parameters, can affect the growth and fatty acid profile of different microalgae. Physicochemical factors include nutrient composition, pH, salinity, etc. whereas light, temperature, and CO_2 are among the environmental parameters (Gatamaneni et al. 2018; Santin et al. 2021).

3.1 Nutrients

Nutrient depletion is commonly used to promote the accumulation of lipids in microalgae. For instance, the amount of nitrogen can be depleted to enhance the production of lipids in microalgae. On the other hand, it can result in a decrease in growth and biomass. The nitrogen is added in the medium culture in the form of nitrate, nitrite, urea, and ammonium, where the nitrate is widely used due to being more stable (Yaakob et al. 2021). However, urea as a nitrogen source for microalgae can increase the ω-3 content (Perdana et al. 2021). Studies like (Rodolfi et al. 2009) showed that the lipid content in *Nannochloropsis* spp. increased from 32% to 60% by having a more nitrogen-deficient condition. Furthermore, the fatty acid composition varies a lot under nitrogen deprivation compared to normal conditions, such as an increase in SFA and MUFA and a decrease in PUFA (Qiao et al. 2016). However, in some other studies (Chen et al. 2011), it has been concluded that the Nitrogen limitation increases the PUFA and ALA levels, but the increases were not more than 5%.

Phosphorus limitation is also employed to increase lipid accumulation. Phosphorus is a part of many important molecules, such as phospholipids, DNA, RNA, and ATP, which makes it an essential nutrient. Microalgae can take up phosphorus in the form of polyphosphate or orthophosphate. During phosphorus starvation, the microalgae store lipids by switching photosynthetic carbon partitioning toward more energy-rich molecules like lipids which blocks starch synthesis (Yaakob et al. 2021). Moreover, phosphorus limitation increases the concentration of ω-6 PUFA by stimulating the activity of Δ6-desaturase, which is one of the responsible enzymes for palmitic acid conversion to LA (Su et al. 2016).

3.2 pH

pH can impact microalgae in many ways, such as changing membrane permeability, affecting nutrients and molecule uptake, altering photosynthetic electron transport, and modifying protein function. The pH ranges from 5-10.6 among microalgae for growing. In general, a lower pH can increase cell growth but restricts lipid production whereas a higher pH can have the opposite effect (Perdana et al. 2021). For instance, in a study (Bartley et al. 2014), the highest lipid content and growth rate for *Nannochloropsis salina* were obtained at pH 8-9. However, the fatty acid composition was not affected very much.

3.3 Salinity

Salinity is another factor that should be taken into consideration when it comes to increasing the lipid content (Chu 2017). The most notable impacts of salinity stress on microalgae are higher lipid content and lower biomass (Singh et al. 2018). Osmotic stress increases glycolysis metabolism, which decreases the carbohydrate content (Wang et al. 2018). The ω-3 PUFA content is not directly affected by salinity stress but the content of SFA increases, due to having a higher energy-storing capacity. For instance, the fatty acid profile did not change by a large margin in salinity up to 25 mM NaCl in *Chlamydomonas americana* (Perdana et al. 2021). However, desaturation is employed in some microalgae to maintain the fluidity and permeability of the cell membrane (Shetty et al. 2019). Some studies revealed that the ALA, EPA, and DHA content was enhanced by 20-300% in *C. vulgaris* and *Dunaliella salina* (Rismani & Shariati 2017). However, the effects of salinity on growth and lipid accumulation are species-dependent (Chen et al. 2017).

3.4 Temperature

Temperature is one of the more important environmental parameters that can significantly influence the fatty acid profile of microalgae. PUFAs are used to maintain cell fluidity and permeability under lower temperatures, which increases the ratio of PUFAs (Ananthi et al. 2021). An example is the higher EPA ratio in *N. salina* under a lower temperature (17 ºC) compared to the room temperature (21-26 ºC). The cultivation of cold-tolerant species might be a rational choice due to being able to produce more PUFA as a response to lower temperatures. For example, *Chlamydomonas* sp., ICE-L can accumulate PUFAs up to 78% at 0 ºC (Suparmaniam et al. 2023). The optimum low temperature is between 10-20 ºC, at which microalgae facilitate more desaturation processes to produce PUFAs, such as EPA (Perdana et al. 2021). On the other hand, a higher temperature increases the ratio of SFA and decreases PUFA content, like in *Nannochloropsis oculate* (Gaignard et al. 2021). In addition, a lower temperature reduces the growth rate, which results in less content of all lipids, including PUFAs (Santin et al. 2021). In conclusion, it is reasonable to suggest that the influences of temperature on cell growth as well as PUFA production require more studies for individual microalgae species.

3.5 Light

The majority of fatty acids found in microalgae are stored in subcellular oil droplets but they are also a part of the structural components of the cell membrane where they can be involved in the organization of the light-harvesting complex (Yan et al. 2013; Wacker et al. 2016). Changes in light intensity result in changes in fatty acid composition in microalgae as fatty acids can affect the properties of the photosynthetically active membranes or accumulate lipids in the case of excessive carbon fixation (Maltsev et al. 2021). For example, in *Phaeodactylum tricornutum,* less intense light (150 µmol photons m^{-2} s^{-1}) was shown to increase the production of PUFAs by 16%, whereas SFA production was higher in irradiation of 750 µmol photons m^{-2} s^{-1} (Conceição et al. 2020). In another study (Maltsev et al. 2021), *Nostoc spongiform* and *Phormidium corium* were exposed to strong light (500 µmol photons m^{-2} s^{-1}) but the level of UFAs remained the same while the SFA content slightly decreased. It indicates a contradiction when it comes to changes in the fatty acid composition of microalgae under intense irradiation. In general, it can be concluded that at high-light intensity, microalgae produce lipids more in the form of SFA and MUFA, whereas at less intense light, PUFA content (mainly EPA) tends to increase (Maltsev et al. 2021). Various light wavelengths were shown to have different effects on the fatty acid composition of each species. In short, Chlorophyta (green algae) manufacture PUFA in high levels under blue light as a response to ROS formation (Ma et al. 2018; Perdana et al. 2021).

3.6 CO_2

In addition to N and P, an inorganic carbon source (CO_2) is required for the growth of microalgae, especially photoautotrophic ones. A concentration of more than 10% CO_2 may decrease biomass and lipid production due to acidifying the medium in the presence of a high concentration of CO_2. Different microalgae need different levels of CO_2 to have optimum production. For instance, Chlorella spp. tends to tolerate a CO_2 concentration of up to 30%, where the optimum level of CO_2 for greater biomass and lipid production was shown to be at 2-8% (Brindhadevi et al. 2021; Gaignard et al. 2021; Suparmaniam et al. 2023). Furthermore, the UFA content, such as LA also increased with a high CO_2 concentration (10%) in *C. vulgaris* studied by Yasmin et al. (2011). In another study, the effect of CO_2 concentrations (5 and 15%) on several microalgae suggested that, in general, the total fatty acid content was highest at 5% CO_2, but the amount of obtained PUFA slightly decreased (Mudimu et al. 2015). On the other hand, in a study done by Ruangsomboon & Chonudomkul (2022) on *N. oculate*, a higher supplement of CO_2 demonstrated a higher content of both EPA and DHA, with a maximum yield at 6% CO_2. Additionally, in *Microchloropsis gaditana* the PUFA content as well as productivity were increased by using a CO_2 level of 3% (Kareya et al. 2020). All of this data indicates that the effects of CO_2 concentration on the growth and lipid content/composition of different microalgae require more research to maximize the cultivation conditions for a higher yield of fatty acids from each microalgae.

3.7 Genetic Engineering

Genetic engineering techniques are the newest and most encouraging strategy to enhance the PUFA content of microalgae. Metabolic pathway editing often results in genotype transformation, where metabolites such as PUFA content can be increased to make them more suitable for large-scale production (Santin et al. 2022). Numerous transformation methods have been developed, such as overexpressing key enzymes to boost PUFA synthesis, knocking out or downregulating enzymes that may compete with the PUFA pathway, and inducing PUFA biosynthetic genes from other organisms (Grama et al. 2022). For example, overexpression of some endogenous genes in *Schizochytrium* spp. increased DHA and EPA respectively by 81% and 172%. Moreover, the EPA content per cell was higher 5-fold in *T. pseudonana* by knocking down lipase (Ghiffary et al. 2019). Additionally, inducing an elongase enzyme (Δ5) from *Ostreococcus tauri* into *P. tricornutum* genome resulted in an 8-fold increase in DHA yield (Sun et al. 2019).

4. Discussion

This study aimed to offer an overview of microalgae as a potential alternative for producing ω-3 LC-PUFAs, in particular EPA and DHA. Some of the physicochemical and environmental factors influencing the fatty acid content and composition of microalgae were presented. Furthermore, the cultivation, harvesting, extraction, and purification of algal oil were briefly described to give a better understanding of production.

The modulation of nutrients like N and P depletion can enhance lipid production. pH can change nutrient uptake and enzyme activities, where the proper range of pH is species-dependent. Although salinity can increase lipid production, the fatty acid content and composition are not significantly affected. The intensity of light can influence PUFA content, especially in phototrophic cultivation. A lower light intensity and blue light are prone to increasing PUFA levels. A lower T often results in a higher PUFA to maintain cell membrane fluidity. CO_2 is primarily required for photosynthesis, but its effects on the fatty acid profile and the optimal concentration vary a lot for different species. However, a very high concentration of CO_2 can decrease biomass and lipid synthesis.

Despite a higher PUFA and lipid content, manipulating abiotic stress factors, such as low T and high salinity during the early stages of cultivation can result in a lower biomass. One of the strategies that can overcome this problem is a technique called two-stage cultivation (Sun et al. 2018). First, microalgae are cultivated in a well-being condition, because the goal is to maximize growth and biomass accumulation. Then, stresses including low T and high salinity will be employed to stimulate the microalgae to produce more PUFAs. Nevertheless, lowering T can be energy-demanding, which can increase the production cost (Lu et al. 2021).

The method chosen for cultivating depends on many parameters, such as the intensity and duration of light, cultivation time, carbon source, risk of contamination, budget, and more. For instance, heterotrophic and mixotrophic cultivation increase cell density and growth rate, and the cultivation systems for these methods are often closed, which decreases the risk of contamination (Dubey et al. 2024). Moreover, the cost of energy for production is reduced in photoautotrophic cultivation as the need for light is less. Nevertheless, cheaper alternatives are demanded due to the high cost of organic carbon sources (80% of the total cost). For instance, waste products from the biodiesel industry, including glycerol, could be utilized to minimize expenses (Liaqat et al. 2023).

In large-scale production, open-pond is used most of the time because it is the most convenient and cheaper for massive growth, but contamination can be problematic. One of the methods to make this issue more avoidable is salinity stress (Liang et al. 2019; Santin et al. 2021).

A large number of the studies are on the laboratory scale, whereas, in the case of large-scale manufacturing, many variables (e.g., CO_2, nutrients, energy supply, light, weather, and contamination) can change the cultivation conditions (Sun et al. 2018; Perdana et al. 2021). Thus, investigations into the workability and profitability of large-scale production are still required.

The challenges during harvesting, including low cell density and the cell wall breakdown of microalgae during drying, and lipid extraction, show the difficulties of production since some crucial points need to be taken into account, such as efficiency and gentleness, to reduce the degradation of lipids. Additionally, the extraction solvents are also required to be inexpensive, easily removable, and free from toxic or reactive impurities to minimize the degradation of lipids (Medina et al. 1998).

The employment of genetic engineering to increase PUFA content in microalgae has presented many opportunities, but some areas still require improvement. For instance, the genome editing toolbox is not as advanced as it needs to be due to the complexity of microalgal cells (e.g., tough cell walls and intron-extron interaction). In addition, the number of cases where genetic engineering was done or successful is small (Ghiffary et al. 2019; Sun et al. 2019). This shows that a better understanding of gene modification and the metabolism of microalgae is necessary. The majority of studies are also conducted by using single-gene overexpression and deletion, which creates uncertainty about the effectiveness of editing more than one gene in microalgae (Sun et al. 2019). Furthermore, public acceptance of genetically modified microalgae is also one of the major challenges that can determine its integration into the food and feed market (Khozin-Goldberg & Sayanova 2019). Thus, long-term risk assessment is demanded to ensure that the genetically modified microalgae is safe for both consumption and the environment.

In this context, microalgae have several benefits that can make them a great substitute for producing PUFAs, particularly ω-3 LC-PUFAs. These benefits include the ability to grow on land that is not suitable for agriculture, the ability to grow at a faster rate, the lack of need for pesticides and herbicides, the requirement for a minimal amount of nutrients for production, and the ability to cultivate in harsh environments for land plant (Barros de Medeiros et al. 2022). Additionally, some can possess a high content of single ω-3 LC-PUFA and antioxidant compounds while being free from potentially toxic agents usually found in fish (Harwood 2019; Fawcett et al. 2022). This shows big opportunities for developments in algal oil production. however, several key challenges remain unaddressed, including low biomass and lipid production, the content of PUFA, bioprocessing time, and economic downstream (Dubey et al. 2024). The cultivation conditions and downstream processing for maximum yield in large-scale production of microalgae still need to be well-studied to develop more cost-efficient manufacturing (Santin et al. 2021).

In conclusion, microalgae are suggested as a sustainable solution for PUFA production. They boast advantages, but currently, they face limitations. Lower yields and inefficient extraction methods make large-scale production expensive. Additionally, optimizing the PUFA ratios within microalgae requires further research. Despite these hurdles, advancements in cultivation techniques, downstream processing, and potentially even genetic modification hold promise for a more efficient and cost-effective approach. By addressing these challenges, microalgae can become a viable and sustainable source of ω-3 fatty acids, meeting the growing demand.

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