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Abstract

Long chain polyunsaturated fatty acids (LC-PUFA) are essential to humans and needs to be consumed through the diet instead of production in the body. LC-PUFAs are in this report be categorized into omega-3 and omega-6 fatty acids. The aim of this study was to focus on LC-PUFAs that can be produced by species of microalgae of commercial interests.

There are estimated to exist 200 000 up to millions species of microalgae and the species are as diverse in cultivation strategies as in products. Strains of *Arthrospira* produce γ-linoleic acid (GLA). *Crypthecodinium* species and *Schizochytrium* species produce docosahexaenoic acid (DHA). *Nannochlorpsis* species, *Nitzschia* species and *Phaeodactylum* species can be cultivated for the production of eicosapentaenoic acid (EPA). *Porphyridium* species are potential for the production of arachidonic acid (AA).

The LC-PUFAs are important due to a wide range of health beneficial services. EPA and AA are the most important producers to eicosanoids among the fatty acids. Eicosanoids are important in the process of cell-signalling and also respond to inflammations. Categories of eicosanoids are prostaglandins, leukotrienes and thromboxane's that are included in blood pressure reductions, pro-inflammatory effects, aggregation of thrombocytes and artery contractions et cetera.

Major depression disease and Alzheimer disease are suggested to benefit from supplements of LC-PUFA. EPA and DHA are part of membranes in the brain, and can improve cognitive functions, hence, have preventative effects to Alzheimer and major depression disease. Cardiovascular disease may also be prevented if sufficient omega-3 fatty acids are consumed. Eicosanoids derived from LC-PUFA may also affect the inflammatory response in the body. Eicosanoids derived from AA have a more severe inflammatory response, while eicosanoids derived from EPA and DHA can have anti-inflammatory response.

Keywords: Micro algae, LC-PUFA, Eicosanoids

Sammanfattning

Långkedjiga fleromättade fettsyror (LC-PUFA) är essentiella för människan vilket innebär att de måste förtäras via dieten eftersom en produktion i kroppen inte är möjlig. LC-PUFA kan i den här rapporten kategoriseras in i 2 huvudgrupper; omega-3 och omega-6. Syftet med denna litteraturstudie var att fokusera på LC-PUFA som kan produceras av olika arter av kommersiellt intressanta mikroalger.

Det beräknas att existera 200 000 till miljontals arter av mikroalger i världen. Arterna är lika mångfaldiga i sin artskildhet som i odlingsstrategi och i sammansättning. Stammar av *Arthrospira* producerar γ-linolensyra (GLA). Arter av *Crypthecodinium* och *Schizochytrium* är potentiella för odling av dokosahexansyra (DHA). Arter av *Nannochloropsis*, *Nitzschia* och *Phaeodactylum* är potentiella för odling av produktion av Eikosapentaensyra (EPA). Arter av *Porphyridium* kan potentiellt odlas för produktion av arakidonsyra (AA).

LC-PUFA konsumtion är viktig på grund av sina utbredda hälsoförtjänster. EPA och AA är de viktigaste producenterna bland fettsyrorna till en rad olika eikosanoider. Eikosanoider är viktiga i processer så som cellsignalering och ger gensvar på inflammation. Det finns olika kategorier av eikosanoider så som prostaglandiner, leukotriener och tromboxaner. Dessa ingår i funktioner som blodtryckssänkning, trombocytaggregering, kärlsammandragningar et cetera.

LC-PUFA kan påverka, enligt studier, hälsan hos sjuka individer. Depressionssjukdomar och Alzheimers har studier föreslagit att motverkas på grund av intag av LC-PUFA. EPA och DHA utgör delar av fosfolipider och membran i hjärnan samt kan förbättra kognitiva funktioner. Hjärt- och kärlsjukdomar kan också förebyggas om omega-3 fettsyror konsumeras, enligt studier. Eikosanoider ursprungligen från LC-PUFA kan även påverka den infammatoriska responsen i kroppen. Eikosanoider från AA har en rikligare inflammatorisk respons medan eikosanoider från EPA och DHA kan ha antiinflammatorisk respons.

Nyckelord: Mikroalger, LC-PUFA, Eikosanoider

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Abbreviations

AA Arachidonic acid
ALA α-Linolenic acid
COX Cyclooxygenase
D-GLA Dihomo γ-linoleic acid
DHA Docosahexaenoic acid
EPA Eicosapentaenoic acid
GLA γ-Linolenic acid
LA Linoleic acid
LC-PUFA Long chain polyunsaturated fatty acid
PUFA Polyunsaturated fatty acid

1 Introduction

Algae are organisms that include a wide range of species that perform approximately 50% of the photosynthesis on the globe (John, 1994). Algae may be macroalgae or microalgae which in both categories includes diversities. Microalgae, also called microphytes, are in focus in this report. In contrast to macroalgae are microalgae smaller (Olaizola, 2003).

The concept of microalgae is wide, and contain all organisms that are small enough (a few µm), unicellular and can mostly live in environments with water. The organism may also be photosynthetic and can be eukaryotic or prokaryotic. This gives opportunities to a wide range of commercial applications such as human nutrition, cosmetics and aquaculture and all are not yet explored (Olaizola, 2003).

The number of species of microalgae that exist are unknown, though estimated to between 200 000 up to millions, while higher plants are estimated to be about 250 000 species (Pulz & Gross, 2004).

Diatoms are a category of microalgae of great importance that fixate about 20 % of the carbon on the planet (Kroth *et al.*, 2008). Blue-green microalgae or cyanobacteria are a category which microalgae show many similarities with, though cyanobacteria are bacteria not algae (SMHI, 2010) and have been categorized as plants due to the photosynthetic properties, but are now called bacteria due to that no defined nucleus exist (Ziboh, 2001).

Microalgae are at the lowest level in the food chain and a source to long chain fatty acids. This give rise to a diversity of wild life in the oceans (Posten & Walter, 2013). All species of microalgae are not suitable for human consumption. Some species have multiple ecosystem favours while some species constitute a risk through algae blooming. Thereby, the algae may toxify the surroundings for fish and wildlife. Humans that consume toxified shellfish or fish may be poisoned (SMHI, 2011).

A few species of microalgae have been used as food supplement and as enrichment in feed for centuries. Accustomed examples are *Nostoc* species in Asia and *Spirulina (Arthrospira* species) in Mexico and Africa (Olaizola, 2003).

In comparison to how many species of algae that exists, only a few species are used since a production needs to be in a large scale, the metabolite of interest needs to be found and purified and the process needs to be financially sustainable (Olaizola, 2003).

Arthrospira species, *Chlorella* species, *Dunaliella* species, and *Aphanizomenon* species are the four strains that are dominating the commercial industry of microalgae as human nutrition. The microalgae act as supplements and natural food colorants. Since there are such a wide range of species among microalgae, their biofunction and products are equally different. Due to studies *Arthrospira* species produce primarily long chain polyunsaturated fatty acids (LC-PUFA), *Dunaliella* species produce primarily β-carotene, *Chlorella* species produce primarily chlorophyll and *Aphanizomenon* species promote a good health in general (Spolaore *et al.*, 2006).

According to the variance in biodiversity, bioavailability and products from the species of microalgae, their applications are also different. Microalgae are used for aquaculture, biofuels, cosmetics, human nutrition, supplements et cetera (Olaizola, 2003).

Apart from the four major genus, other important microalgae used for commercial applications are *Chaetoceros* species that are used for aquaculture and indirect consumption. *Crypthecodium* species and *Schizochytrium* species are used for docosahexaenoic acid-oils (DHA-oils) and *Nannochloropsis* species are used for aquaculture feed (Spolaore *et al.*, 2006).

Since the 1930’s it has been known that fat is necessary for a good health. Linoleic acid (LA, 18:3, n-6) and α-linolenic acid (ALA, 18:3, n-3) were supplemented to e.g. cure people diseased with different dermatological problems et cetera (Abrahamsson *et al.*, 2013).

The human body cannot synthetize fatty acids (FA) with double bounds on carbon 3 and 6. In different studies, different names rise to longer more unsaturated fatty acids such as; long chained polyunsaturated fatty acids (LC-PUFA), essential fatty acids (EFA) and polyunsaturated fatty acids (PUFA) (Wiktorowska-Owczarek *et al.*, 2015).

LC-PUFAs are essential since the body cannot synthetize them. Two important categories of LC-PUFA are omega-3 and omega-6 FA. Omega-3 FA can be consumed in the diet in the form of ALA and can then be transform to longer omega-3 fatty acids such as docosahexaenoic acid (DHA) and/or eicosapentaenoic acid (EPA) in the body. Omega-6 FA can be consumed in the diet in the form of LA that can be transformed into the omega-6 fatty acids γ-linolenic acid (GLA) and arachidonic acid (AA). The importance of these LC-PUFAs are their beneficial health effects (Livsmedelsverket, 2015b).

Algae are a source to LC-PUFAs, either through purification of the algae into oils, such as DHA-oil, through enrichment of food with dried biomass or through use in aquaculture (Spolaore *et al.*, 2006). Potential ways to use algae for LC-PUFA production are also the use of algae genes, inserted in other crops (Kris-Etherton & Hill, 2008).

The aim of this literature analysis was to describe 7 genuses of microalgae that are used or potentially can be used in the market of human nutrition for their production of LC-PUFA (Harwood & Guschina, 2009). The aim was to answer questions concerning if the conditions of cultivation of microalgae can affect the nutritional value of LC-PUFA and if microalgae is a possible source in comparison with other sources. The functions that the LC-PUFA provide for the human body will also be elucidated.

2 Method

Databases such as Web of Science, Scopus, Primo, PubMed and Google Scholar were used in the literature analysis. In first hand, scientific journals and review articles were studied which referred to more profound scientific publications. The scientific publications were chosen by reading the abstract and then those of interest were read more thoroughly. Many sources were dated and therefore as updated sources as possible were used primarily, though some dated books were used for background facts. Books were searched through the database Primo and were viewed and studied online.

The databases from National Food Agency of Sweden (Livsmedelsverket) were used and also the S wedish Meteorology Authority's website (SMHI).

1 Long chained polyunsaturated fatty acids

In this report the focus is on long chain polyunsaturated fatty acids (LC-PUFA) as a product of 7 genuses of microalgae that are cultivated, or has potential to be cultivated for this purpose (Harwood & Guschina, 2009). Arachidonic acid (AA, 20:4 n-6, 9, 12), γ-linolenic acid (GLA, 18:3 n-6, 9, 12), eicosapentaenoic acid (EPA, 20:5 n-3 6, 9, 12, 15) and docosahexaenoic acid (DHA, 22:6 n-3 6, 9, 12, 15,18) are LC-PUFAs that could be connected as a product of the different algae species selected (Spolaore *et al.*, 2006). The chemical structure of the essential LC-PUFAs can be seen in Table 1 (Appendix).

The omega 3 and omega 6 fatty acids are essential for humans, since the human body cannot create double bounds on carbon 3 and 6 in the fatty acid chains (Wiktorowska-Owczarek *et al.*, 2015). This is due to lack of the enzymes Δ12- and Δ15-desaturase which inserts the double bounds on the n-3 and n-6 positions. Therefore, these fatty acids need to be consumed through the diet (Abrahamsson *et al.*, 2013).

Linoleic acid (LA, cis-9,12-octadecenoic acid) is the original omega-6 fatty acid that can be consumed in different food such as; maize-oil, sunflower-oil, rapeseed-oil, sesame-oil. AA and GLA are also omega-6 fatty acids, deriving from LA, that can be developed with help from desaturases and elongases (Livsmedelsverket, 2015b). Desaturases are enzymes that are necessary to form double bounds and different elongases are needed to create longer fatty acid chains (Wiktorowska-Owczarek *et al.*, 2015).

α-Linolenic acid (ALA, cis-9,12-octadecatrienoic acid) is the original omega-3 fatty acid that can be consumed in different food such as walnuts, rapeseed-oil, fatty fish and algae. DHA and EPA can be developed from ALA, hence includes to be omega-3 fatty acids. There are also other omega-6 and omega-3 fatty acids whilst AA is the most occurring in the omega-6 family and DHA and EPA are the most occurring in the omega-3 family (Livsmedelsverket, 2015b). The metabolism of the LC-PUFAs in the human body can be seen in Table 2 (Appendix).

Challenges concerning dietary intake of omega-3 and omega-6 fatty acids are that they compete over the same enzymes in the elongation and desaturation process (Gebauer *et al.*, 2006). Therefore, LC omega-6 FA is more easily produced in the body, since its more abundant in the diet (Gebauer *et al.*, 2006). Though, omega-3 fatty acids have higher affinity to the enzymes (Abrahamsson *et al.*, 2013). Omega-9 fatty acids are also included in this competition (Kinsella *et al.*, 1990).

1.1 Arachidonic acid

AA has an important role to play in the human body to create cell membranes to integrate the cells and also to produce prostaglandins. The concentration of AA in the body tissue is correlated to the dietary intake of LA up to 3 E%. This is the point when the phospholipid membranes in the body are saturated with AA.

There are different ways AA can affect the human body. Lipase enzymes are necessary to decompose AA from the phospholipid membranes. To create the prostaglandins D, E and F, the cyclooxygenase (COX) enzymes are necessary. In the cardiovascular tissue an instable prostaglandin I_2 (PGI_2) can form by COX and in the thrombocytes, thromboxane A_2 (TXA_2) can form. Another pathway for AA is with lipoxygenase enzymes that converts AA to hydroxyl fatty acids, leukotrienes and lipoxins (Abrahamsson *et al.*, 2013).

AA is the most common occurring long chain fatty acid and owns the competition of lineolate enzymes over EPA and DHA. It is important for humans to consume both omega-6 and omega-3 fatty acids since it is necessary for a normal health (Livsmedelsverket, 2015b). Studies show that an elevation of plasma level of AA is not often desirable. The accumulation of thromboxane's and platelet aggregating peroxidases can have pro-inflammatory effects (Barham *et al.*, 2000).

1.2 γ-Linolenic acid

GLA is an omega-6 fatty acid that is the first product that is produced in the body from the dietary intake of LA (Livsmedelsverket, 2015b), commonly from vegetable oils. GLA is necessary for the omega-6 metabolism and is created in the first desaturation step with Δ6-desaturase. GLA is then quickly transformed into dihomo-γ-linolenic acid (D-GLA) by a polyunsaturated fatty acid-elongase. D-GLA can be transformed further into AA by Δ5-desaturase. The desaturase enzymes are membrane bound and needs aerobic conditions (Ziboh, 2001).

In the body GLA creates D-GLA that together with AA and EPA is the primary sources to eicosanoids in the body (Sergeant *et al.*, 2016).

A wide range of diseases, such as i.e. cancer and diabetes, have negative impact on the development from LA into GLA. Inhibited initial pathway to omega-6 fatty acids can be solved through supplements of GLA. By passing the first Δ6-desaturaton step, LC-PUFA may be created and instead have beneficial health effects on the diseased. This has been applied in many medicinal purposes (Ziboh, 2001). Even though, supplements of GLA are in some aspects discussed due to that the GLA that is not transformed to D-GLA, can accumulate the plasma levels of AA. High plasma levels of AA can in turn have pro-inflammatory properties (Barham *et al.*, 2000). Besides, D-GLA do not create any prostaglandin or thromboxane of importance (Abrahamsson *et al.*, 2013).

1.3 Eicosapentaenoic acid

EPA is an omega-3 fatty acid that can be transformed in the body from ALA in the diet. ALA is often rich in fish, shellfish, rapeseed et cetera (Livsmedelsverket, 2015b).

EPA has important health influencing effects on humans. EPA is an important source to eicosanoids in the body together with AA and D-GLA and can synthetize the prostaglandin PGI_3, that is similar to PGI_2, that has cardio vessel dilating effects (Abrahamsson *et al.*, 2013).

Since omega-6 FA is more abundant in the diet of the western society than omega-3 fatty acids, approximately in 10:1 ratio, this may lead to that AA is more abundant in membranes and tissues of the human body (Parker *et al.*, 2006). Omega-3 fatty acids have a higher affinity to the elongating enzymes in the body and can inhibit the synthesis of AA, and be more abundant in the body (Abrahamsson *et al.*, 2013).

EPA produce the prostaglandin PGI_3 that has similar effects as PGI_2 from AA, while thromboxane from EPA has no crucial effect (Abrahamsson *et al.*, 2013).

1.4 Docosahexaenoic acid

DHA is an omega-3 fatty acid that can be transformed in a minor amount in the body from ALA, which is widespread in the diet. Fish and algae are good sources to DHA and can be consumed directly (Livsmedelsverket, 2015b). Studies claim that the transformation from dietary ALA is insufficient and only occurs into DHA and EPA in 10-15% (Parker *et al.*, 2006). A series of elongation and desaturation steps needs to occur to produce DHA in the human body from ALA (Sergeant *et al.*, 2016).

2 Health effects from LC-PUFA

LC-PUFA create health effects in the human body that may prevent, slow down or improve health (Abrahamsson *et al.*, 2013). Different studies point out that Alzheimer disease, cardiovascular disease, inflammation and major depression disease may have beneficial health aspects from the dietary intake from LC-PUFA (Song *et al.*, 2016; Mori & Beilin, 2004; Kris-Etherton *et al.*, 2003).

Beneficial health effects are due to eicosanoids, derived from fatty acids with 20 carbon atoms, that can control cell signalling and respond to inflammations (Abrahamsson *et al.*, 2013).

Studies have shown that beneficial health effects such as anti-inflammatory response are due to D-GLA (Sergeant *et al.*, 2016). DHA is crucial for a normal brain activity, the nervous system and the cardiovascular system. This is especially during development for a growing and maturing child (Abrahamsson *et al.*, 2013).

2.1 Alzheimer disease

The human brain contains high levels of PUFA. Due to this facts, studies have been performed concerning brain health and its correlation to PUFAs, such as EPA and DHA (Logan, 2003).

Alzheimer disease is a brain disease which has no cure and only have a few options for treatment (Swanson *et al.*, 2012). LC-PUFA may be preventative factors since the brain benefit from their functions. EPA and DHA have important functions in membranes, inflect the neuroimmune and apoptotic pathways and also compete over enzymes with omega-6 fatty acids that may have pro-inflammatory effects (Song *et al.*, 2016).

Studies have shown that neurological functions have improved in correlation with a high intake of DHA in proportion to EPA and that patients with a mild form of Alzheimer might benefit from supplements of DHA and EPA through e.g. improved cognitive functions (Swanson *et al.*, 2012).

2.2 Cardiovascular disease

Studies have shown that people with cardiovascular disease (CVD) can have beneficial health effects from supplements or food rich in omega-3 fatty acids, such as fish.

Intervention studies imply that high blood pressure can be partially reduced, and that high fat compared to low fat and enhanced LA have blood pressure reducing effect. Blood coagulation, aggregation of thrombocytes and extended bleeding time are functions dependent on DHA and EPA (Abrahamsson *et al.*, 2013).

Three epidemiological prospective studies showed that grown men that had an intake of fish more than once a week had decreased risk for coronary heart diseases (Kris-Etherton *et al.*, 2003). The aspect to how women respond to the dietary intake of fish has been concluded to lower risk for coronary heart disease, according to "The Nurses' health study", (Hu *et al.*, 2002) performed over 16 years. Though, a number of prospective cohort studies on women showed that atherosclerosis could benefit from the dietary intake of fish, which was contradictory to the studies done in men (Hu *et al.*, 2002).

2.3 Inflammation

Eicosanoids from omega-6 fatty acids are suggested to create inflammation if high dietary intake occur. Eicosanoids derived from AA are proposed as even more severe in the inflammatory response which can be modulated by EPA and DHA derived eicosanoids (Abrahamsson *et al.*, 2013).

In the aspect of anti-inflammatory response, the EPA and DHA omega-3 fatty acids from marine-oil are effective (Mori & Beilin, 2004).

Cytokine production and development of lymphocytes and reactive oxygen radicals are all pro-inflammatory markers, that can be inhibited by eicosanoids derived from EPA and DHA. Therefore, immunological diseases, psoriasis, hypersensitivity, lymphocytes, rheumatism and intestinal diseases have been suggested to be prevented from EPA and DHA (Abrahamsson *et al.*, 2013).

The anti-inflammatory effects deriving from EPA and DHA are shown in trials, preventing atherosclerosis, sudden death, myocardial infarction et cetera (Mori & Beilin, 2004). Studies show that high dietary intake of trans fatty acids and saturated fatty acids in comparison to alpha linolenic acid give rise to pro-inflammatory markers. Exceeding 10 E% of PUFA can give rise to peroxides in the body which can be reduced by vitamin E in the diet (Abrahamsson *et al.*, 2013).

2.4 Major depression disease

Major depression disease (MDD) is a common illness of the brain. Approximately 10% of the population in the world is affected and gets depressive, downhearted, unmotivated, indifferent and apathetic (Song *et al.*, 2016).

Studies have shown that the lipid levels in the bloodstream are less dense in people with MDD or similar diseases, and more abundant in healthy people. Research emphasize the importance of LC-PUFA to the central nervous systems which can be preventative to various of neuropsychiatric disorders (Logan, 2003).

Other studies show connection between MDD and stress, which give rise to dysfunctional hormone levels in the brain and results in low amount of neurotransmitters such as dopamine, serotonin and noradrenaline (Song *et al.*, 2016). Other studies show that post-traumatic stress from pregnancies can have beneficial effects, if treated with LC-PUFA (De Vriese *et al.*, 2003).

3 Microalgae species and LC-PUFA

The microalgae species that are in focus in this report are *Arthrospira* species, *Cryptecodium* species, *Nannochloropsis* species, *Nitzchia* species, *Phaeodatylum* species, *Porphyridium* species and *Schizochytrium* species. These species were chosen in this report due to their production of LC-PUFA; AA, GLA, EPA and DHA (Harwood & Guschina, 2009). The microalgae are used in products in the market or are potential industrial products. (Posten & Walter, 2013).

Strategies to increase LC-PUFA content in microalgae are well studied. Examples of strategies are the possibility of optimization of temperature. The light intensity can be altered, as well as nutrients, the concentration of carbon dioxide, the pH and the salt concentration. There are also more factors that can be included to demonstrate that there are many different ways to alter the composition of the microalgae (Cavonius, 2016). The fatty acid content of the microalgae genuses seen in Table 1. in Appendix is modified by a study and describes the content of DHA and EPA in percent of the dried biomass and the yield DHA and EPA in milligram per litre of the total batch cultivation.

Studies results in different values of the total lipid content of microalgae genuses. In a study the lipid composition of different microalgae genuses was determined by thin-layer chromatography-flame ionization detection. The lipid classes the microalgae species contained were hydrocarbons, triacylglycerols, free fatty acids, sterols and polar lipids (Volkman *et al.*, 1989).

Microalgae yield more oil than conventional crops due to that microalgae use the vegetable oil as storage product, and contain about 50-60% lipids per dry weight of the total biomass (Griffiths & Harrison, 2009). Approximated value of LC-PUFA of the total lipids in percent is given in Table 2. in Appendix.

3.1 *Arthrospira* species

Arthrospira species are blue-green microalgae, which in the market for human consumption are also called *Spirulina*. *Spirulina* is categorized to be a microalgae, but are really spiral shaped dietary consumable cyanobacteria (Vonshak, 1997).

Arthrospira species have long been studied for its fatty acid composition (Muhling *et al.*, 2005). Food supplement oils from *Arthrospira* species can provide rich amounts of GLA. The total lipid content is in general 6-7% of the dried biomass of *Spirulina maxima* (Spolaore *et al.*, 2006).

Due to one study on S. *plantensis* the content of PUFA was 18.6%, monounsaturated fatty acids (MUFA) 1.9% and saturated fatty acids (SFA) 70.3% of the total lipid content. GLA was the major fatty acid of the PUFA (18.5%) followed by LA (13.8%). Also existing were palmitic acid, palmitoleic acid and oleic acid (Yasar & Sevket, 2006).

Arthrospira species are suitable for cultivating for human consumption since they are rather large in comparison with most microalgae, rich in protein and easily can be digested. The production of GLA can be altered by growth conditions such as light intensity, growth phase and temperature. Studies show that the growth condition in 30-35 °C for most strains of *Arthrospira* is richest in GLA. High light intensity and temperature reduces fatty acid content but not composition (Cohen *et al.*, 1987). *Arthrospira* species have been suggested to have an optimized growth under light and dark cycles in laboratory environment or outdoors (Tanticharoen *et al.*, 1994).

3.2 *Crypthecodinium* species

Crypthecodinium cohnii is a heterotrophic microalga that is grown for food and pharmaceutical interests for the production of DHA-oil. Studies has been made on growth conditions to optimize the production of DHA-oil. One study used batch cultivation with sea salt, yeast extract and glucose and compared with another batch with acetic acid as carbon source. The batch with acetic acid showed high biomass, DHA and lipid concentrations (De Swaaf *et al.*, 2003).

Crypthecodinium species are studied to produce DHA (Spolaore *et al.*, 2006). A recent study determined the DHA and EPA proportion of the dried biomass of *Crypthecodinium cohnii*. The results showed that it contained 0.8-1.3% DHA and 0% EPA of the dried biomass and measured in milligram fatty acids per litre of batch culture, DHA reached 15-20 mg/L and EPA 0 mg/L (Cavonius, 2016). Hence, that *Crypthecodinium cohnii* almost exclusively produces DHA makes it a good microorganism for purification process of lipids (Mendes *et al.*, 2009).

3.3 *Nannochloropsis* species

Nannochlorpsis species are green microalgae that are cultivated for their chlorophyll, carbohydrate, protein and fatty acid content and some *Nannochloropsis species* also produce LC-PUFA (Volkman *et al.*, 1989). Some species of *Nannochlorpsis*, have been shown to produce EPA (Spolaore *et al.*, 2006). In a study, *Nannochlorpsis* species grown under enriched CO_2 phototrophic culture conditions gave a higher yield of EPA compared to acetate enriched mix trophic conditions (Hu & Gao, 2003).

Nannochloropsis occulta is composed of 17.8-39.9% EPA of the total fatty acid content (Renaud *et al.*, 1991). A recent study determined the DHA and EPA proportion of the dried biomass of *Nannochlorpsis* species. The results showed that it contained 0-0.7% DHA and 0-8.2% EPA and contained 0-2.6 mg/L DHA and 0-14 mg/L EPA in the total batch culture (Cavonius, 2016).

3.4 *Nitzschia* species

Nitzschia laevis is a diatom (Chen *et al.*, 2007), which means that the microalga contain silicon in the cell wall (SMHI, 2011).

Nitzschia species have been confirmed to produce EPA (Spolaore *et al.*, 2006). In a study *Nitzschia laevis* showed that the EPA production was most sufficient in a mix trophic growth culture compared to using single trophic conditions (Wen & Chen, 2000). *Nitzschia laevis* is a potential for production of EPA and the distribution of EPA has been shown to be spread among the classes of lipids. In one study regarding *Nitzschia laevis*, the total lipid content constituted 78.6% neutral lipids. Out of these neutral lipids, 87.9% was triacylglycerols and out of the triacylglycerols, 75.9% was EPA (Chen *et al.*, 2007).

A recent study determined the DHA and EPA proportion of the dried biomass of *Nitzschia* species. The results showed that it contained no detected DHA and 1.1-1.5% EPA and the total batch culture contained no detected DHA/EPA in mg/L (Cavonius, 2016).

3.5 *Phaeodactylum* species

Phaeodactylum tricornitum are diatoms that are potentially used for the production of EPA-oil for omega-3supplement. *Phaeodactylum* species have been confirmed to produce EPA (Spolaore *et al.*, 2006). A study showed that the cultivation of *Phaeodactylum tricornitum* have higher production of EPA and PUFA at slightly lower temperatures than at higher temperatures (Jiang & Gao, 2004).

In another study of the fatty acid composition, it was found that the EPA content was high in the strains studied. The highest amount of EPA was 25.65% of the total lipids (Wu *et al.*, 2016). A recent study determined the DHA and EPA proportion of the dried biomass of *Phaedactylum tricornutum.* The results were 0-0.4% DHA and 0.2-5.5% EPA and the total batch culture contained 0-11 mg/L DHA and 1.3-130 mg/L EPA (Cavonius, 2016).

3.6 *Porphyridium* species

Porphyridium species are red marine microalgae that produce the LC-PUFAs AA and EPA. AA is of special interest since it is unusual to be produced (Cohen *et al.*, 1988). A study showed that slow growth conditions and nitrogen starvation on *Porphyridium cruentum* resulted in the highest values 2.9% AA in ash free dry weight and 2.4% EPA of total lipids in ash free dry weight (Cohen, 1990). Microalgae are produced for DHA-oils and EPA-oils as food supplements (Spolaore *et al.*, 2006). The *Porphyridium* species produce AA and have potential to be directly consumed (Spolaore *et al.*, 2006).

To be able to yield the production of AA by *Porphyridium* species it is suggested that the light intensity should be 8000 lux and the temperature 32℃ in liquid cultures of 12 x 109 cells/L (Ahern *et al.*, 1983). A recent study determined the DHA and EPA proportion of the dried biomass of *Porphyridium* species. The results were 0% DHA and <0.1-3.8% EPA and the total batch culture contained 0 mg/L DHA and <1-69 mg/L EPA (Cavonius, 2016).

3.7 *Schizochytrium* species

Schizochytrium species are thraustochytrids, which means algae-like fungi (Ziboh, 2001). *Schizochytrium* species are marine microheterotrophic organisms, which has been detected to produce high biomass containing LC-PUFA. Though, these LC-PUFA profiles are rather complex and that yields production costs. Thraustochytrids with simpler LC-PUFA profiles are preferable in a production point of view (Lewis *et al.*, 1999).

Schizochytrium species produce DHA (Spolaore *et al.*, 2006). The *Schizochytrium* species strain 31 can improve production of DHA when the cultures are incubated during 4 days with initial of pH 7.0, with 0.4% (w/v) yeast extract and with 20 g/L glucose (Wu *et al.*, 2005).

Schizochytrium mangrovei isolated from decaying mangrove forest in Hong Kong was studied for fatty acid content and DHA was found to be the most abundant fatty acid. The percentage DHA was 32.29-39.14% of the total fatty acid content (Jiang *et al.*, 2004). A recent study determined the DHA and EPA proportion out of the dried biomass of *Schizochytrium aggregatum.* The results were 0-

0.3% DHA and 1.0-1.2% EPA and the total batch culture contained 0-<1 mg/L DHA and 6.1-9.3 mg/L EPA (Cavonius, 2016).

4 Discussion

Many studies have shown that the composition of microalgae can be altered due to different culture conditions. Different strains in each species prefer different conditions and some strains produce more LC-PUFA than others (Wu *et al.*, 2005; Hu & Gao, 2003; Jiang *et al.*, 1999; Renaud *et al.*, 1991).

Fish oil is used as the main source for supplements of EPA and DHA, and studies show this to be successful(Parker *et al.*, 2006; Kris-Etherton *et al.*, 2003).

Fish industries have concerns regarding sustainability though microalgae may be an alternative. Microalgae are also superior to crops that accumulate omega-3 FA, in the aspect of cultivation space, maturation time and higher growth rate (Mercer & Armenta, 2011).

Studies have shown that fish accumulate toxins and pollutants which have given an increased interest in microalgae as supplements in replacement (Mimouni *et al.*, 2015). Pregnant women that are especially sensitive for the safety for their fetus are recommended to limit their dietary intake of some fish (Livsmedelsverket, 2015a).

A downside to microalgae cultivation is the use of water. The large amount of water in the algae culture requires cheap energy methods to dry the biomass (Cavonius, 2016).

The separation methods that are used to release lipids into organic solvents from microalgae are not environmentally sustainable. Methods, to be able to use microalgae as an environmental alternative to gain LC-PUFA is under research and one study shows that it is possible to take advantage of the solvent characteristics and use pH shifting to extract soluble material (Cavonius, 2016).

Some algae might be better than others depending on what kind of LC-PUFA that is of interest. Though, the quota of EPA and DHA that is most beneficial is not possible to determine. The total amount of dietary intake of EPA and DHA is more important for a good health (Livsmedelsverket, 2015b). Microalgae supplement may be a good source to LC-PUFA as long as common food is not neglected

(Livsmedelsverket, 2015c). Fish is a good source within reasonable ranges (Livsmedelsverket, 2015a).

Microalgae are a good source to LC-PUFAs if cultivated in financially sustainable systems. Microalgae are only discussed superficially due to the existing thousands of species. Microalgae need less cultivations space than plants and fishery ponds and produce high biomass. Microalgae yield about 50-60% dry weight lipids of the total biomass which is more oil production than conventional crops due to that microalgae use the vegetable oil as storage product.

In comparison with how many species of algae that exists, only a few species are used since a production needs to be in large scale and the metabolite of interest needs to be found and purified to be financially sustainable.

LC-PUFA are essential for humans and can in some cases be needed for supplements, especially for target groups. LC-PUFA is needed for the infant's development, pregnant women also need to keep this in mind, hence algal supplement may be suitable. Microalgae (wet mass) do not contain pollutants and toxins in same range as fish products.

The correlation between the dietary intake of omega-3 and omega-6 fatty acids is more important than the actual amount out of each category, while the optimal correlation is not set.

There is need for more controlled clinical trials with more subjects to be able to draw more conclusions considering microalgae. It is crucial to distinguish socio-demographic variables such as gender, age et cetera in the trials. This is due to the fact that LC-PUFA is more important during different stages in the maturation phases and also to different individuals.

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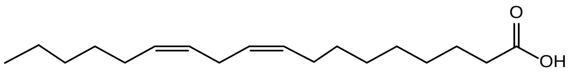
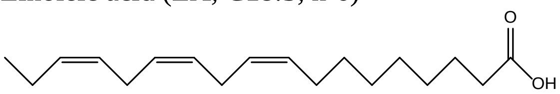
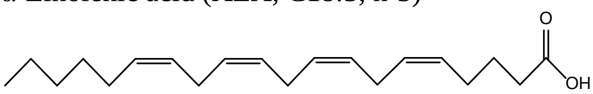
Appendix



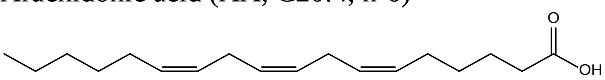
Linoleic acid (LA, C18:3, n-6)



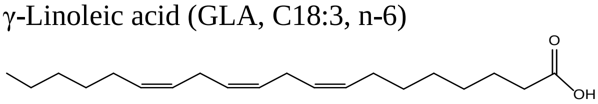
α-Linolenic acid (ALA, C18:3, n-3)



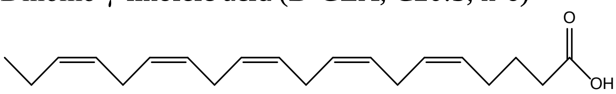
Arachidonic acid (AA, C20:4, n-6)



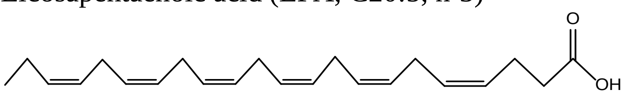
γ-Linoleic acid (GLA, C18:3, n-6)



Dihomo γ-linoleic acid (D-GLA, C20:3, n-6)



Eicosapentaenoic acid (EPA, C20:5, n-3)



Docosapentaenoic acid (DHA, C22:6, n-3)

Figure 1. Chemical structures of LC-PUFA, modified from: (Abedi & Sahari, 2014).

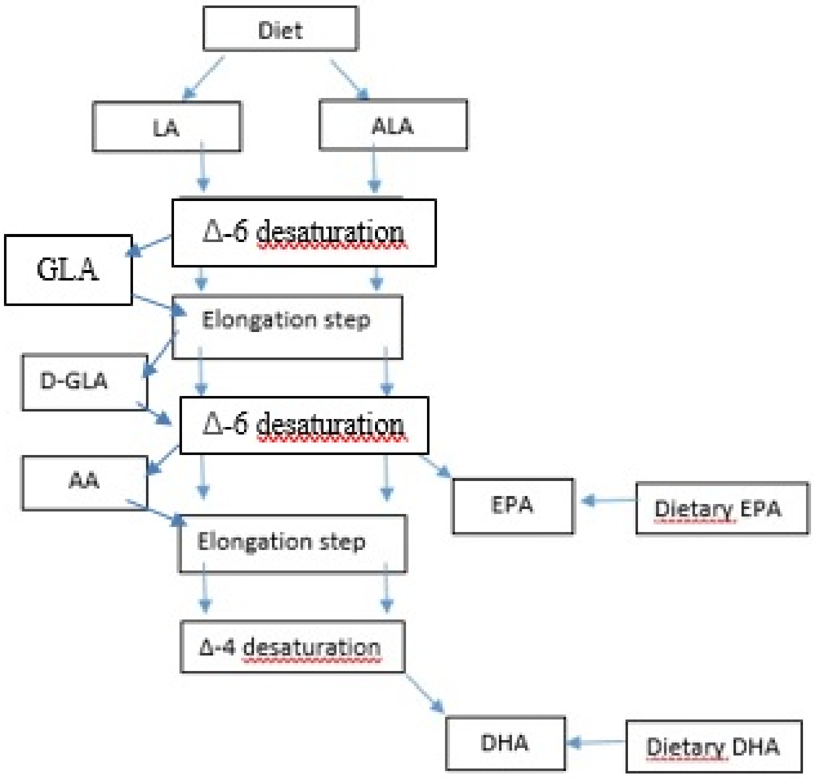


Figure 2. Metabolism of essential fatty acids in the human body. ALA= α-linolenic acid, LA= Linoleic acid, D-GLA= Dihomo γ-linoleic acid, AA= Arachidonic acid, EPA= Eicosapentaenoic acid, DHA= Docosahexaenoic acid. Modified by: (Kris-Etherton & Hill, 2008).

Tabell 1. Microalgae used for human nutrition and the production of DHA and EPA of dried biomass in % and the yield of fatty acids of batch cultivation in mg/L. Modified from Cavonius, 2016.

Genuses	**DHA (%** of dried biomass)	**EPA (%** of dried biomass)	**DHA (mg/L)**	**EPA (mg/L)**
Cryptecodinium cohnii	0.8-1.3	0	15-20	0
Nannochlorpsis species	0-0.7	0-8.2	0-2.6	0-14
Nitzschia species	Not detected	1.1-1.5	Not detected	Not detected
Phaeodactylum tricornutum	0-0.4	0.2-5.5	0-11	1.3-130
Porphyridium spe-cies	0	<0.1-3.8	0	<1-69
Schizochytrium aggregatum	0-0.3	1.0-1.2	0-<1	6.1-9.3

Tabell 2. Microalgae species used for human nutrition. The approximated value LC-PUFA of the total lipids and other approximated FA values. DHA= docosahexaenoic acid, EPA= eicosapentaenoic acid, MUFA= monounsaturated fatty acids, NL= neutral lipids, SFA= saturated fatty acids, TAG= triacylglycerols ((1) Cavonius, 2016; (2) Wu et al, 2016; (3) Chen et al, 2007; (4) Jiang et al, 2004; (5) Cohen, 1990; (6) Yasar & Sevket, 2006).

Genuses	**Approximated value LC-PUFA of total lipids (%)**	**Other approximated FA values and lipids**
Arthrospira species	18.6 (7)	1.9% MUFA, 70,3% SFA of total lipids (6)
Crypthecodinium species	30-50 (1)	0.8-1.3% DHA, 0% EPA of dried biomass (1)
Nannochloropsis species	0-8.2 (1)	0-0.7% DHA, 0-8.2% EPA of dried biomass (1)
Nitzschia species	-	75.9% EPA of 87.9% TAG of 78.6% NL of total lipids (3)
Phaeodactylum species	25.6 (2)	0-0.4% DHA, 0.2-5.5% EPA of dried biomass (1)
Porphyridium species	0- 2.9 (5)	0% DHA, <0.1-3.8% EPA Of dried biomass (1)
Schizochytrium species	32.2-39.1 (4)	0-0.3% DHA, 1.0-1-2% EPA Of dried biomass (1)