Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Molecular Sciences

Algae and Polyunsaturated Fatty Acids

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Independent project • 15 credits

Agricultural Programme - Food Science
Molecular Sciences, 2019:4

Uppsala 2019

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Credits:	15 credits
Level:	First cycle, G2E
Course title:	Independent project in food science
Course code:	EX0876
Course coordinating department:	Department of Molecular Sciences
Place of publication:	Uppsala
Year of publication:	2019
Title of series:	Molecular Sciences
Part Number:	2019:4
Online publication:	https://stud.epsilon.slu.se
Keywords:	Algae, polyunsaturated fatty acids, EPA, DHA, omega-3

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Abstract

The human body cannot produce enough amounts of long chain polyunsaturated fatty acids (LC-PUFA), on its own, to sustain the biological functions they have. Consuming essential fatty acids is fundamental for well-functioning bodily processes. The essential fatty acids are linoleic acid (LA) and alpha-linolenic acid (ALA). ALA is the precursor to the LC-PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The metabolism of EPA and DHA in the human body is very limited and it is therefore important to have these fatty acids in our diet. The essential fatty acids can be found in some nuts, seeds and vegetable oils, they are most prominent in canola oil and linseed oil. EPA and DHA cannot be found in nuts, seeds or vegetable oils but are instead found in algae and fish.

ALA, EPA, and DHA are categorised as omega-3 fatty acids. Omega-3 fatty acids have an important role in the cell membrane and in retinal tissue. The omega-3 makes the cell membrane fluid and flexible, facilitates the cellular functions and cell signals. EPA is a precursor for the hormone like biochemical substances called eicosanoids. Eicosanoids help aid multiple bodily functions, such as immune functions and inflammatory responses as well as blood pressure regulations and muscle activity. The health benefits of consuming PUFAs are many. They have shown to aid both mental and cardiovascular health. Visual functions have shown risks of being compromised when PUFAs were limited during the infant years. In algae, EPA and DHA are maintaining the photosynthetic functions and may aid cell signalling.

Algae have been utilized for over thousands of years. Asians have been the primary consumers, but the consumption of algae is spreading all over the world. Algae are the base, the lowest trophic level, in the aquatic food chain. In modern day they are primarily used as food, but they are also used as biofuel and to purify waste waters. Not all algae are high producers of PUFAs, the most common ones, nori, spirulina etc., are richer in protein and nutrients. The *Schizochytrium spp* are microalgal species rich in DHA. *Phaeodactylum tricornutum* and *Odontella aurita* are two microalgae rich in EPA. The aim of this literature study was to introduce PUFAs and their biological functions. The study also covers the role algae have in the production of EPA and DHA.

Keywords: PUFA, algae, omega-3, DHA, EPA

Sammanfattning

Människokroppen kan inte producera tillräckliga mängder av långa fleromättade fettsyror för att upprätthålla de biologiska funktioner fleromättade fettsyror har. Konsumtionen av essentiella fettsyror är central för välfungerande biologiska processer. De essentiella fettsyrorna är linoleic acid (LA) och alfa-linolenic acid (ALA). ALA är prekursor till de längre fleromättade fettsyrorna eicosapentaenoic acid (EPA) och docosahexaenoic acid (DHA). Människokroppens metabolism av EPA och DHA är väldigt begränsad, därför är det viktigt att dessa finns med i vår diet. De essentiella fettsyrorna finns i vissa nötter, frön och vegetabiliska oljor. Raps- och linfröolja är exempel på livsmedel rika på essentiella fettsyror. EPA och DHA finns inte i dessa livsmedel utan hittas främst i alger och fisk.

ALA, EPA, och DHA kategoriseras som omega-3 fettsyror. Omega-3 har en viktig roll i cellmembranen och i retinal vävnad. Cellmembran med omega-3 är mer flexibla och fluida, det hjälper cellulära funktioner och cellsignalering. EPA är en prekursor till de hormonliknande ämnena eikosanoider. Eicosanoider hjälper flera kroppsliga funktioner, såsom immunologiska funktioner och inflammatoriska reaktioner. De hjälper också till att reglera blodtryck och muskelaktivitet. Det finns flera hälsofördelar med att äta fleromättade fettsyror. De har visat förbättra mental- och kardiovaskulär hälsa. Brist på fleromättade fettsyror under spädbarnsåldern har visat sig rubba vissa visuella funktioner. I alger hjälper EPA och DHA till med att underhålla fotosyntetiska funktioner och eventuellt också cellsignalering.

Alger har använts i flera tusen år. Asiater har varit de primära konsumenterna men en spridning har skett över hela världen. De används främst som livsmedel men kan också används som biobränsle och för att rena vatten. Inte alla alger producerar höga halter fleromättade fettsyror. De vanligaste, nori och spirulina med mera, har högre halter av protein och andra näringsämnen. Arter av mikroalgen *Schizochytrium* har höga halter av DHA. *Phaeodactylum tricornutum* och *Odontella aurita* är två exempel på arter rika på EPA. Syftet med denna studie var att introducera fleromättade fettsyror och deras biologiska funktioner. Studien tar också upp algers roll i produktionen av EPA och DHA.

Nyckelord: fleromättade fettsyror, alger, omega-3, EPA, DHA

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Abbreviations

ALA	α-Linolenic acid; 18:3n3
AA	Arachidonic acid; 20:4n6
DHA	Docosahexaenoic acid; 22:6n3
DPA	Docosapentaenoic acid; 22:5n6
EPA	Eicosapentaenoic acid; 20:5n3
GLA	γ-Linolenic acid, 18:3n6
GPL	Glycerophospholipid
LC	Long chain
PUFA	Polyunsaturated fatty acid

1 Introduction

Polyunsaturated fatty acids (PUFAs) have an important biological role in the human body. They take part in maintaining a healthy brain function and coronary health (Ander *et al.* 2003), as well as giving our cells special characteristics to function properly (Hishikawa *et al.* 2017). PUFAs are divided into omega-3 and omega-6 fatty acids, where omega-3 fatty acids are less common in the western diet than omega-6 fatty acids. The ratio between these two have been widely researched and it was believed to be 1:1 in our ancestors' diet. For the modern diet, many different ratios have been recommended over the years. The most common one being a 4:1 ratio, or less, of omega-6 and omega-3 (Bhardwaj *et al.* 2016).

According to study made by The Swedish National Food Agency in *Riksmaten – 2010-2011: Livsmedels- och näringsintag bland vuxna I Sverige* (Amcoff *et al.* 2012), the Swedish population acquire more omega-3 today than they used to. Swedes who eat a varied diet obtain 2,7 g omega-3 per day. To keep a regular intake of omega-3, The Swedish National Food Agency recommend a diet that consist of fish two-three times a week, where at least one of them is a fat fish e.g. mackerel and salmon etc. Though most swedes eat a healthy amount of omega-3, most western diets do not consist of enough omega-3 (Martins *et al.* 2013). For people who do not eat fish, for example vegetarians, vegans and people with fish allergies, is it important to obtain the omega-3 from other sources (Livsmedelsverket 2018). Other foods rich in omega-3 are vegetable oils, nuts and seeds (Francieli da Silva *et al.* 2015)

The most important omega-3 fatty acids are alpha-linolenic acid (18:3n3; ALA), eicosapentaenoic acid (20:5n3; EPA) and docosahexaenoic acid (22:6n3; DHA). The omega-6 fatty acids are linoleic acid (18:2n6; LA), gamma-linolenic acid (18:3n6; GLA), arachidonic acid (20:4n6; AA) and docosapentaenoic acid, (22:5n6; DPA). ALA and LA are essential fatty acids and cannot be synthesised in the human body. The other PUFAs are synthesised in a very limited degree in the human body and are therefore also considered as essential in our diet (Ward & Singh 2005).

The importance of obtaining PUFAs during the first infant years have been studied extensively. DHA is present in all brain tissue, in some areas up to 40%. Due to its vital role, DHA is now a staple component in infant formula. Several studies suggest that DHA benefits the eye development and is critical for the brain to develop (Happe & Gambelli 2015).

There is no doubt that PUFAs are important for human health and development. Fish is the most common and important source, but the fish stocks are depleting and new sources for PUFAs needs to be found. Algae are one of these new sources that could be a future staple food in the everyday diet.

1.1 Aim

The aim of this thesis is to summarise some of the current knowledge of algae rich in PUFAs. An introduction to PUFAs and the biological importance they have will be covered, as well as give the current prospects of PUFAs. To justify the interest in research about algae rich in PUFAs, one must also know the important biological role PUFAs have.

1.2 Method

Information for this literature study have been fund by searching on *PubMed*, *ASFA: Aquatic Sciences and Fisheries Abstracts, FSTA (Food Science and Technology Abstracts), Primo* and *NCBI*. The searches were made with the following words in different combinations. *Microalga**, alga*, marine phytoplankton, omega-3, omega-6, essential, fatty acid, PUFA, LC-PUFA, EPA, DHA, neurological, health, biologic*, function, role, synthes*, metabolism, pathway, carotenoid*, astaxanthin, cultivat*, food chain, food, biofuel, Odontella, Schizochytrium, Phaeodactylum tricornutum.*

2 Polyunsaturated Fatty Acids

2.1 Definition

Polyunsaturated fatty acids (PUFAs) are all fatty acids with more than one double bond in either a cis or trans configuration. The long chain - polyunsaturated fatty acids (LC-PUFAs) are the fatty acids longer than 20 carbons. The omega-3 fatty acids have a double bond on the third carbon from the methyl end on the carbon chain and omega-6 fatty acids have the double bond located on the sixth carbon from the methyl end (Wallis *et al.* 2002).

The most abundant PUFA in mammalian tissue is DHA. It is synthesised of 22 carbon atoms and six double bonds, often specified as 22:6n-3 or 22:6ω3. The double bonds in DHA are all in cis configuration (Brenna & Carlson 2014). The second most abundant PUFA is EPA. EPA consists of 20 carbons and 5 double bonds, also these are in cis configuration. Precursor for both these fatty acids is ALA.

AA and DPA are two other PUFAs in mammalian tissue and require LA to be synthesised (Wallis *et al.* 2002).

2.2 Essential fatty acids

Though the western diet is overflowing with omega-6 fatty acids, one of them is essential for us. The true essential fatty acids are linoleic acid and alpha-linolenic acid. LA is an omega-6 fatty acid and ALA is an omega-3 fatty acid. LA can be produced by plants and is the backbone for the omega-6 fatty acid family including GLA, AA and DPA (Pasquale 2009). ALA is also produced by plants and it is the precursor for the omega-3 family containing EPA and DHA.

2.3 Dietary sources

There are many plant-based- and marine foods that contain PUFAs. Mackerel, salmon, seabass sardines, trout and shrimp all contain high amounts of DHA and EPA. Supplements such as fish oil, cod liver oil and krill oil are also rich in DHA and EPA. Algae oil is also on the market but is more expensive and does not contain as high amounts of DHA and EPA as the other supplement oils. Users of algae oil therefore need to take more and the costs will be higher (Gal 2018).

Chia-, hemp- and flax seeds are rich in ALA, but do not contain any DHA or EPA. Walnuts as well as edamame, kidney beans and soybean oil are also rich in ALA (Gal 2018).

Most vegetable oils are rich in LA (Pasquale 2009). ALA is not as common but can be found in canola oil (rapeseed oil) and linseed oil (flax seed oil). Linseed oil is the vegetable oil most abundant in ALA, it can contain up to 65% ALA. However, because of the high amount of unsaturated fats, it is not suitable for cooking and frying. Excessive heating of the oil can lead to auto-oxidation of the double bonds, giving an off-flavour and causing polymerization of the fatty acids (Kolodziejczyk *et al.* 2012).

2.4 Metabolism of PUFAs

Humans cannot synthesise their own omega-3 fatty acids, we are dependent on eating plants and algae — or consumers of algae — to maintain our intake. Though we cannot synthesise them, omega-3s are essential for our bodies to function properly. EPA and DHA can be synthesised from ALA to a limited extent in our cells, but it is not enough to sustain our bodies. The same goes for the essential omega-6 linoleic acid and its metabolism to AA (Wallis *et al.* 2002).

Plants synthesise the omega-3 fatty acid ALA from the saturated fatty acid steric acid (18:0). Steric acid (18:0) is desaturated with Δ9 desaturase to oleic acid (18:1), which in return is desaturated with Δ12 desaturase to linoleic acid (18:2n6). Linoleic acid then undergoes another desaturation with n3 desaturase to synthesise alpha-linolenic acid. The Δ12- and n3 desaturases are enzymes lacking in humans, this is what makes ALA an essential fatty acid and must therefore be obtained in the diet.

ALA is considered one of the most important omega-3 fatty acids, because by using ALA mammalian cells can, to some extent, synthesise EPA and DHA. The ALA is elongated and desaturated in multiple steps by Δ6- and Δ5 desaturases and elongation enzymes in the endoplasmic reticulum to tetracosahexaenoic acid (24:6n3). A chain-shortening β-oxidation of the tetracosahexaenoic acid then occurs in the peroxisome, converting it to DHA (Wallis *et al.* 2002).

Similarly, the omega-6 LA is desaturated with a $\Delta 6$ enzyme in the human body. It is then elongated and desaturated once again by $\Delta 5$ desaturase to form AA (Wallis *et al.* 2002).

The limited production of EPA and DHA in the human body, makes the consumption of fish and algae extra important. Algae are big producers of EPA and DHA, and since algae are the base of the aquatic food chain, the omega-3 fatty acids will accumulate in the fish as well.

2.5 Algal biosynthesis

Most marine organisms are believed to utilise the polyketide synthase pathway intertwined with the fatty acid synthesis to produce LC-PUFAs such as EPA and DHA. This is a complex machinery and requires approximately 30 distinct enzyme activities and nearly 70 reactions to synthesise DHA from acetyl-coenzyme A (Metz *et al.* 2001). The entire process is complex and is not yet fully understood.

3 Biological role of LC-PUFA

3.1 Omega-3

The research of EPA and DHA is extensive. Some research focus on their biological functions and the mechanisms contributing to health. DHA and EPA take part in multiple biological functions in the human body. One of the main functions is the part in glycerophospholipid (GPL), which is the main component of the cell membrane. DHA can be one of the two lipids in the GPL molecule. The cis double bonds in DHA give the GPL a specific characteristic, that contributes to many biological functions (Hishikawa *et al.* 2017).

DHA-containing GPLs affect the membrane fluidity, membrane flexibility, the lipid packing defect and the membrane thickness.

DHA-containing GPLs contributes to a highly fluid cell membrane that may allow the cellular functions that are dependent on a fluid membrane to work better (Hishikawa *et al.* 2017). The higher fluidity can impact on the receptor migration in the cell membrane as well as lipid raft formation. It can also modify cell signalling pathways that by extension impact cell and tissue responses linked to metabolism, hormone sensitivity and immunological functions (Walker *et al.* 2015). It may also promote membrane fusion and fission that are dependent on the membrane's flexibility, as well as promote conformational changes and the mobility of membrane proteins (Hishikawa *et al.* 2017).

The lipid packing defect refers to the exposure of the GPL's hydrophobic tail in strongly curved membranes. When the GPL contains a DHA, the exposure is limited because of the cis curved carbon chain. Membranes that consist of many DHA-containing GPLs are thinner compared to those with GPLs containing more saturated fatty acids. The thinner membrane increases the permeability of small polar molecules (Hishikawa *et al.* 2017).

Apart from cell membrane functions the DHA-containing GPL also has an important role in tissues. In the retina, the DHA-containing GPL promotes rapid conformational changes in the rod photoreceptors after light stimulation, which allows the eye to adjust better between light and darkness as well as stimulate night vision (Hishikawa *et al.* 2017).

DHA and EPA replace other fatty acids such as AA in the phospholipids in the cell membrane. This alters the balance between eicosanoid and cytokine production in the body. This promoting a less inflammatory or even inflammatory resolving profile instead of the previous generally pro-inflammatory profile (Walker *et al.* 2015). EPA can also be metabolised to eicosanoids. Eicosanoids are hormone-like biochemical substances that aid multiple bodily functions very effectively. Cell growth, cell division, muscle activity, regulation of blood pressure, immune functions and inflammatory responses are all affected by these eicosanoids. Eicosanoids derived from EPA may help protect against heart diseases, strokes and different inflammatory diseases (Bhardwaj *et al.* 2016).

3.2 Omega-6

The consumption of too many omega-6 fatty acids are believed to increase all inflammatory diseases. Diseases such as cardiovascular disease, type 2 diabetes, irritable bowel syndrome, asthma and cancer are all believed to have increased in correlation to the increase of omega-6 in our diet. The omega-6 fatty acids compete with omega-3 fatty acids for the use of the same conversion enzymes in our bodies. LA is converted to AA and will indirectly limit the conversion of ALA to EPA and DHA. An overconsumption of omega-6 can therefore interfere with the positive functions of omega-3 (Bhardwaj *et al.* 2016).

From a physiological point of view, AA is the most important omega-6 fatty acid. It as a major component in membrane phospholipids, building up most of the cell membrane (Dumancas *et al.* 2012). AA can, just like EPA, be metabolised to eicosanoids, but this process is not a preventive reaction. Instead, they are released in the body as a response to an injury, infection, stress or certain diseases causing an inflammation (Dumancas *et al.* 2012; Bhardwaj *et al.* 2016).

3.3 Health benefits

Essential fatty acid deficiency is an issue getting bigger with the modern western diet. Not only, are the essential fatty acids decreasing in our diet with the choices of food we eat, but they are also decreasing in the food itself. The modern large-scale production and growth of food decreases the overall essential fatty acids contained in the food. The main issue being the diet we feed our livestock, poultry and fish with. Grains are not as essential fatty acid rich as leafy plants and grass. Animals that roam free contain roughly seven times more omega-3 fatty acids than commercially raised meat (Pasquale 2009).

Studies have shown that LC-PUFAs are important for mental health and cardiovascular health. Many studies support that DHA is an important factor when dealing with memory. A consistent intake of DHA have been shown to decrease the risk of memory loss and diseases associated with loss of memory, such as Dementia and Alzheimer’s (Brenna & Carlson 2014).

The need for DHA in infant formulas has been brought to attention in the twenty-first century. Deficiency of DHA during infant years have shown risks of compromised visual function (Brenna & Carlson 2014). A deficiency in omega-3 fatty acids, in correlation to increasing consumption of omega-6 fatty acids, have shown signs of higher risks of cardiovascular disease (Block *et al.* 2008). A regular intake of 3g/day of omega-3 fatty acids have shown a decrease in blood pressure. Most significant in individuals older than 45 years (Mori 2017).

3.4 PUFA function in algae

The role of LC-PUFAs in algae is not fully understood. One of them is believed to be a contribution to the photosynthetic function. In the thylakoid membranes, LC-PUFAs are a part of the galactosyl glycerides: sulfoquinovosyl diacylglycerols (SQDG), monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG). In the green alga *Chlamydomondas reinhardtii*, SQDG have been shown to uphold photosystem II during environmental changes, such as temperature (Sato *et al.* 2003). They have also been known to interact with reactive oxygen, when exposed to light. This lipid peroxidation process may play a role in cell signalling pathways as an intermediate (Mühlroth *et al.* 2013).

3.5 Carotenoids

The high lipid content in microalgae would not be possible without carotenoids. Carotenoids are antioxidants preventing lipid peroxidation in biological membranes. They do this by removing high-energy electrons from free radicals. Astaxanthin is one of these carotenoids, it is also a red pigment responsible for the red colour in salmon and shellfish (Gammone *et al.* 2015). Astaxanthin has a very strong antioxidative effect and is considered the most effective antioxidative compound (Galasso *et al.* 2017). It is ten times more efficient than lutein, canthaxanthin and β-carotene and 100 times more than α-tocopherol (Sun *et al.* 2011).

Astaxanthin and other carotenoids also have the same beneficial antioxidative role in humans. They scavenge free radicals in our body and quench singlet oxygen activity (Gammone *et al.* 2015).

Astaxanthin is most abundant in the microalgae *Haematococcus pluvialis*. *H. pluvialis* is therefore commonly used for industrial cultivation of astaxanthin (Galasso *et al.* 2017).

4 Algae

4.1 Algae as a base of the aquatic food chain

The main dietary source of LC-PUFAs is fatty fish and algae (van Ginneken *et al.* 2011). One of the reasons fish is rich in LC-PUFA is because they eat algae or other organisms that eat algae. Fish consumption per capita has increased from 9,0kg in 1961 to 20,2kg in 2015, and the population growth is from 3,075 to 7,349 billion (Food and Agriculture Organization of the United Nations 2018). With the increasing population growth and fish consumption, the fish stocks are diminishing. Alternative sources of LC-PUFAs are therefore needed.

The aquatic food chain starts with, as in every other food chain, a primary producer. This is a low living organism that utilise photosynthesis for energy and to build carbohydrates. In the aquatic food chain, phytoplankton, algae and bacteria are on the lowest trophic level. These organisms synthesise their own energy by photosynthesis or chemosynthesis (National Oceanic and Atmospheric Administration 2019). Chemosynthesis is the process of generating energy and organic compounds without sunlight, instead by oxidation of inorganic or C-1 organic molecules. These molecules could be hydrogen gas, hydrogen sulphide, ammonium, methane or methanol etc. (Enrich-Prast *et al.* 2009).

The second trophic level consists of zooplankton — including copepods and amphipods as well as the egg and larval stages of some animals — and larger animals, such as marine snails, fish, reptiles and mammals. Zooplankton mostly graze on phytoplankton and the larger animals graze on algae. Bivalves, sponges, whales and manta rays are examples of filter feeders that strain plankton directly from the water for food.

Predators are next in the food chain, here we find the many shark, jellyfish and fish (National Oceanic and Atmospheric Administration 2019). The accumulation process makes the predators accumulate nutrients, fatty acids and more in their tissue. Though fish can have an abundance of omega-3, it would not be possible without algae and other LC-PUFA producing organisms.

4.2 Different uses of algae

The industry of cultivating algae is growing every day, it could be a lucrative market and there are plenty of future opportunities within this field. The cultivation of algae does not compete with already established agriculture and creates job opportunities for our growing population.

Algae have some advantageous characteristics that make them useful in many industries. Algae grow fast and consume CO_2, nitrogen and phosphorous (Sharma & Sharma 2017). They can grow in saline waters and would therefore not be leaching on our already depleting fresh water supply. They can also tolerate different pH levels and temperatures making them adaptable to all kinds of waters (Khan *et al.* 2018). The algae can be used to purify wastewaters from nitrogen and phosphorous compounds and turn it into biofuel (Singh & Das 2014). The microalgal biomass can be used as fuel, feed and food. The fatty acids can be extracted to create a vegan omega-3 supplement. Macroalgae are already consumed in many cultures all over the world, they are energy rich and contain many nutrients, bioactive compounds and essential fatty acids (Wells *et al.* 2017).

Algae are energy rich and have high biofuel yields (Sharma & Sharma 2017). The use of algae as biofuel is getting more popular with our growing population and the increasing demand for fuel energy. Microalgae have high energy levels with rich sources of polysaccharides and lipids. The microalgae grow fast and fixate CO_2, which make the fuel both eco-friendly and non-toxic. Microalgae have been reported to fix 1,83 kg of CO_2 with 1 kg of algal biomass and 50% of dry weight consists of CO_2. The technology to produce algal fuels is yet to be improved, but shows on a promising future development (Khan *et al.* 2018).

4.3 Algal foods

Algae have been a part of the human diet all over the world for thousands of years (Spolaore *et al.* 2006). One of the current macroalgae used as food is *Porphyra* species. *Porphyra* spp. have a high protein content (<47%) and they are widely cultivated, it has more than 100 species located all over the world. It goes by many names and is known as “nori” in japan and is a staple ingredient used in sushi. In the United Kingdom it is commonly called “laver”, “karengo” in New Zealand, “zicai” in China, and “kim” in Korea. In Japan, Korea and China it is one of the largest aquaculture industries. The increasing popularity in *Porphyra* is leading the industry to spread to other Asian countries (Baweja *et al.* 2016).

Palmaria palmata is commonly known as “dulse” or “dilisk”. It grows on the coastline of the North Atlantic Ocean. The alga is eaten both raw and dried in Ireland (Seaweed.ie 2019). The alga is also a popular snack in Nova Scotia, Canada, where it is eaten dried (Bay of Fundy 2011). *Palmaria palmata* has a protein content of about 35% and also contains vitamin B12 (Baweja *et al.* 2016).

Arthrospira platensis, commonly called spirulina, is a dietary supplement existing both in powder form and in a tablet (Burgess 2018). It is rich in protein, approximately 65% of dry weight, and is also rich in vitamins, essential amino acids and minerals. It is one of the few sources containing the fatty acid GLA (Belay 2002). Spirulina has become a popular super food and is widely used all over the world, it is a common supplement in influencers’ smoothies and nut-bars.

Another algal food is agar, it is generally used in baking as a substitute for gelatine. It is extracted from some red algae species, most commonly *Gelidium* and *Gracilaria*. Agar is a polysaccharide part of the cell wall of red algae (Doty *et al.* 1983).

4.4 Some examples of microalgae rich in LC-PUFAs

4.4.1 Schizochytrium spp.

Schizochytrium spp. are unicellular eukaryote microalgae part of the *Thraustochytriaceae* family. They are known for producing high amounts of DHA, this by converting sugars to omega-3 (Simris 2019). The species can be found growing all over the world. From the cold waters of Antarctica to the sub-tropical waters of Hong Kong and Australia (Jiang *et al.* 2004). Multiple studies have been conducted to determine the DHA content in *Schizochytrium* spp. and the result have varied. The *Schizochytrium* spp. have been documented to produce between 25-56% DHA of the total fatty acids (Martins *et al.* 2013). Ward & Singh (2005) have recorded one strain to contain more than 94% DHA of the total fatty acids. In addition to the high levels of DHA amongst the fatty acids, the *Schizochytrium* spp. also have an overall high lipid concentration. Containing about 50% lipids of biomass dry weight (Nakahara *et al.* 1996). They are also fast growers and have high resistance to mechanical stirring in fermenter culture as well as a high salinity tolerance (Martins *et al.* 2013). In an industrial cultivation *Schizochytrium* spp. can produce up to 7,2 g of DHA per litre of culture per day (Mühlroth *et al.* 2013).

4.4.2 Phaeodactylum tricornutum

One of the most EPA abundant microalgae is *Phaeodactylum tricornutum*. The unicellular diatom can exist in three different forms: fusiform, triradiate, and oval. It is fast growing and produces EPA from sunlight (Simris 2019). Studies of the total EPA fatty acid content vary in a range between 28-57% of the total fatty acids. The total EPA fatty acid dry weight content in the alga range between 1-5% (Martins *et al.* 2013).

P. tricornutum is a well-studied alga. It can be found as different strains all over the world. From the warmer waters of Micronesia to Long Island, NY to the cooler waters of the Baltic sea (Martino *et al.* 2007). *P. tricornutum* have shown qualities of adaptive growth in unstable environments, such as estuaries and rock pools and in a wide range of salinity. It have also adapted to cultivate in different climates, from a more tropical strain that grow in 20-26 °C to strains living in much cooler climate where the water freezes in the winter (Martino *et al.* 2007).

According to research made by Yongmanitchai & Ward, multiple factors affect the production of EPA in *P. tricornutum*. Temperature, nitrate concentration, phosphate concentration, sodium chloride concentration as well as pH, vitamins and oleic acid additions all have an effect on the total EPA production (1991).

Another alga producing EPA is *Porphyridium cruentum. P. cruentum* is a member of the Rodophyta, Red alga, phylum. It is a single cell alga that is rich in both AA and EPA (Ahern *et al.* 1983; Martins *et al.* 2013)

4.4.3 Odontella aurita

Odontella aurita is a diatom with high a production level of EPA (25-26% of total fatty acids). It is an approved food supplement (Haimeur *et al.* 2012) and has been cultivated for commercial uses, as a dietary supplement, for many years. As with all algae, stress influences the amount of PUFAs produced. Temperature is one of them and in the study by Pasquet et al. (2014) they investigate the impact it has on the PUFA productivity by the alga. They also measure the differences in PUFA at different growth phase.

The temperatures they investigated were 8, 16 and 24°C and they harvested in the exponential- and stationary growth phase. When cultivated at 8°C the diatom grew slower but produced more LC-PUFAs and less saturated fatty acids than when cultivated in 16 and 24°C. The maximum cell density was about five and six times lower than in 16 and 24°C. The percentage of LC-PUFAs were more abundant in the stationary phase than in the exponential phase. When cultivated in 16 and 24°C the growth rate and final cell count were relatively similar, but the abundance of

LC-PUFAs were significantly different. When harvested in the exponential phase, the 16°C *O. aurita* had about seven times more EPA than the 24°C *O. aurita*. When harvested in the stationary phase it was only 1,3 times more. The DHA showed similar patterns and was at its highest when cultivated in 16°C and harvested in the exponential phase. Conclusively, for a maximum amount of cell count and LC-PUFA production, cultivation at 16°C and harvest in the exponential phase is to prefer (Pasquet *et al.* 2014).

In another study made by Xia et al. (2013) they investigate the effects of nutrients and light intensity on *O. aurita*. At the temperature of 25°C, the highest lipid content (19.7% of dry weight) was obtained under 0.11 mmol/L silicon and high light conditions at harvest time. EPA ranged from 9% to 20% of total fatty acids. The harvest phase was not stated (Xia *et al.* 2013).

5 Discussion

Algae have many biological benefits and functions, one of them is the production of LC-PUFAs. The result of this literature study shows the biological advantages of consuming LC-PUFAs. ALA can be metabolised in the omega-3 pathway and synthesise EPA and DHA. EPA and DHA are both synthesised by algae and by eating algae oil, the recommended daily intake can be reached.

The aquatic food chain leads to an accumulation of LC-PUFAs in fish, which have been the main source of LC-PUFAs for human consumption. Early humans were believed to have a more developed synthesis of LC-PUFAs, but their dietary evolution of eating more fish have led to a loss of those enzymes (Mathias *et al.* 2012).

Fish may have been the primary source of LC-PUFAs, but the continuing growth of population have caused the fish stocks to deplete and new sources of LC-PUFAs are needed. The cultivation of algae is still in its early phases and show prosperous development and could be a provider of many jobs in the future. Algal species such as *Schizochytrium*, *Phaeodactylum tricornutum* and *Odontella aurita* are high producers of LC-PUFAs and are vastly researched, but they are not the only ones (Martins *et al.* 2013). Finding the ultimate algae to cultivate could be a challenge since multiple factors play a role in the growth and nutrient production. The final outcome of nutrients is dependent on the environment which the alga is grown in. To cultivate a wide variety of species around the world could be a better path to follow than to just focus on one. This would also support the biodiversity.

In conclusion, research have shown that omega-3 fatty acids are important to our bodies and finding new sources of it is of importance. The fish stocks are depleting, and many diets do not sustain the need for omega-3. Algae are a source rich in omega-3, that can meet the vegan diet restrictions. A substitute to fish oil made from algae is a prospective food supplement that could provide omega-3 to our growing population.

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