Is omega-3 from algae a good substitute to fish oil – in human nutrition?

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Är Omega-3 från alger ett bra substitut till fisk olja – som näring för människan?

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Abstract

Polyunsaturated fatty acids have been proven to be important for human health, especially the omega 3 and 6. Humans cannot synthesise these fatty acids by themselves and need therefore to get them from external sources. Omega-6 is found in many different foods, such as flaxseeds, walnuts, and vegetable oils. In the modern diet, where vegetable oils are used frequently there is no issue in consuming enough omega-6. Consequently, it is creating health problems as the ratio between the two omegas become unbalanced. The optimal ratio should lie between (omega-3: omega-6) 1:3 to 1:5, however this is rarely the case. Studies have shown that high intake of omega 6 and little omega-3 influences the chances of inflammation disorders, cardiovascular diseases, and mental illness, to mention a few.

Discoveries have been made since the 60's showing the health benefits of omega-3, along with the increase of risk for chronic illnesses when there is a lack of this omega. Therefore, the recommendation to consume 250 mg a day has been highly intensified. The demand of omega-3 has been increasing for decades and since fish oil is the main source, it does not have a sustainable future. Thus, there has been a high interest in finding alternatives.

There are different types of omega-3, the three main ones are α- linoleic acid (ALA), Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). ALA is found in many plants on land, whereas DHA and EPA come from marine animals such as fish, krill and algae. ALA can metabolise to DHA and EPA, however, the process is slow and it doesn't reach the levels needed for optimal health. Therefore, direct sources of these two fatty acids are valuable. Algae is a favourable discovery, as it contains high amounts of DHA or EPA, in some cases it can contain both, although it's not often the case.

Several studies have been made to compare the effects of DHA and EPA from algae oil and fish oil in human nutrition. The studies reviewed in this thesis have proven to show that algae oil has similar effect as fish oil. More studies need to be made, to find the ideal species of algae, as there is a huge variety. Identifying more algae species will help to find higher concentrations of DHA and/or EPA, as well as increase the productivity. So far, these are some of the issues that seem to limit this supplement to grow commercially.

Keywords: omega-3, EPA, DHA, algae oil, fish oil, fish oil substitute, PUFA, LC-PUFA, human nutrition, health benefits, algae as n-3 source

Sammanfattning

PUFA har bevisats vara viktiga för människans hälsa, särskild omega-3 och 6. Eftersom människan inte kan tillverka dessa FA måste de komma från mat eller kosttillskott. Omega-6 finns i flera olika livsmedel som linfrö, valnötter eller vegetabiliska oljor. I den moderna kosten, där vegetabiliska oljor används ofta och med stor variation inom olika produkter, är det inga problem att få sin dagliga dos av omega-6. Problemet som uppstår är en obalans mellan de två omegas, som påverkar hälsan negativt. Det optimala förhållandet mellan intag av omega-3 och omega-6 bör ligga mellan 1:3 till 1:5, men det är sällan fallet. Studier har visat att ett högt intag av omega-6 och lite omega-3 påverkar risken av inflammations störningar, hjärt-kärlsjukdomar och mental ohälsa.

Sedan 60 talet har det upptäckts flera hälsofördelar med omega-3, samtidigt som man har sett en ökning av kroniska sjukdomar i de fall där det har funnits brist av omega-3. Detta har förstärkt rekommendationen att inta 250mg omega-3 per dag. Efterfrågan av omega-3 har ökat under flera årtionde och fiskolja är huvudsakliga källan, har den ingen hållbar framtid. Således, finns det ett stort intresse för att hitta ett alternativt.

Det finns olika typer av omega-3, de tre huvudsakliga är α- linoleic acid (ALA), Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). Den första finns i växter på land, däremot DHA och EPA kommer från marina djur så som krill, fisk och alger. ALA kan omvandlas till DHA och EPA, dock är processen väldigt långsam och gör det omöjligt att nå optimala nivåer för god hälsa. På grund av detta, är direkta källor av dessa fettsyror värdefulla. Härmed är alger en gynnsam upptäckt, då de innehåller stora mängder av DHA och EPA, och i vissa få fall kan de innehålla båda.

Flera studier har gjorts för att jämföra effekterna av DHA och EPA från alger och fiskolja i kosten. Studierna som har granskats i denna uppsats har påvisat att olja från alger har liknande effekter som fiskolja. Flera studier behövs för att hitta den ideala alger inom det enorma utbudet som finns. Genom denna identifikation kommer möjligheter uppstå att upptäcka arter med höga mängder av DHA och/eller EPA, och de som kan öka produktiviteten. Detta är viktigt, eftersom detta verkar vara de största faktorer som begränsar denna kosttillskott at växa kommersiellt.

Sökord: omega-3, EPA, DHA, algae oil, fish oil, fish oil substitute, PUFA, LC-PUFA, human nutrition, health benefits, algae as n-3 source

Table of contents

List of figures

Abbreviations

ALA	α- linoleic acid
C	Carbon
COX	Cyclooxygenase
CVD	Cardiovascular disease
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FA	Fatty acids
LC	Long chain
LOX	Lipoxygenases
PhL	Phospholipids
PUFA	Polyunsaturated fatty acids
SDA	Stearidonic acid
TNF-alpha	Tumour Necrosis Factor alpha

1. Introduction

Already in the Roman times fish oil was incredibley popular, in those days it was consumed as Garum, a fish oil-based sauce used for flavouring. Already then it was believed it had medicinal properties. Which in the 1920's had a breakthrough when George and Mildred Burr discovered that fatty acids such as omega-3 were essential nutrients. It took until the late 60's through the 70's to get a better comprehension of the benefits and the composition of omega-3 in fish oil (Harris *et al.* 2021). Today omega-3 from fish oil is an extremely popular supplement, which also creates a problem due to the extended fishing in our seas, causing extinction in some species and disastrous effects in the environment (Lenihan-Geels *et al.*, 2013).

Polyunsaturated fatty acids (PUFAs), are a large group of fatty acids which include omega-3 and omega-6. The latter one is produced by plants on land, such as vegetable oils, which are easily available in the everyday meals eaten, whereas omega-3 is found in marine-food and less likely to be part of most modern days diet. This creates an imbalance in the ratio of these omega's, the optimal ratio should lie between (ω -3: ω – 6)1:3 to 1:5 (Topuz 2016). There is strong evidence that this has led to various health issues that are affecting worldwide, (Topuz, 2016) furthermore that omega-3 has numerous benefits to human health. To mention a few: prevention of cardiovascular diseases, positive effects on inflammatory conditions and it also plays an important role in psychological and psychiatric health in adults, as well as slowing down cognitive disorders in the elderly (Kagan *et al.* 2013).

Humans cannot synthesize omega-3, therefore it needs to come from outside sources, however research shows that it's not so easy to get enough in human diets which has resulted in supplements gaining much attention.

Knowing that fish oil is such an important component in our nutrition, has created an eagerness in finding more sustainable sources. One of the better substitutes found is micro-algae. Being the primary source of omega-3 in the marine food chain, it also contains highest concentration of omega-3 and the least contaminants.

1.1 Research issue

Even so, researching the market of omega-3 supplements, fish oil wins by far. Having looked at different health stores and finding that algae oil is such a minority brings a curiosity to what the reason is and if it is an equivalent substitute to fish oil supplements in human nutrition.

1.2 Aim of the thesis

The aim of this literature study was to research if micro-algae are a good omega-3 substitute to fish oil, in human nutrition. To get a better understanding, background knowledge of polyunsaturated fats as well as its importance in human nutrition has been covered.

2. Method

In this literature analysis different databases were used such as Web of Science, Scopus, Primo, PubMed, Google and Google Scholar. To start, any type of articles on the topic were read, which helped to adjust the search words to find the scientific articles and reviews which has been used for this project.

To justify the aim of this study, a thorough background of the omega-3 needs to be achieved as well as the effects of it. Studies about health benefits and comparative studies were used as references to achieve a conclusion in the differences and similarities between fish oil and algal oil supplements in human nutrition.

The literature search for the comparative studies was at random to receive different perspectives. Regarding the health benefits, a few were chosen which were recurring in my search.

Search words used for this project: *micro-algae omega-3, fish-oil omega-3, omega-3 and human nutrition, Algae as n-3 source, algae as substitute of omega-3, effects of algae omega-3, omega-3 from fish oil vs algae oil, EPA and DHA, functions of EPA and DHA, LC-PUFA, comparative studies fish oil and algal oil, fish oil as n-3 source, effects of algae omega-3 and fish oil omega-3, nutritional value of omega-3*

3. Omega-3

3.1 Polyunsaturated fatty acids

The major component of lipids are fatty acids (FA), containing an aliphatic chain with a carboxylic acid group. Most natural FA contain 14-24 carbons. There are saturated or unsaturated fatty acids, where the latter contain double bonds and they have either cis- or trans configuration. When they contain 2 or more double bonds, they are called polyunsaturated fatty acids (PUFA) (McClements & Decker 2017). However, most common unsaturated PUFAs contain cis configuration, trans configuration are found as intermediates in the biosynthesis of fatty acids such as ruminant fats, plant lipids and some seed oils (Calder 2008).

Fatty acids with chains containing more than 20 carbon- are called long-chain polyunsaturated (LC-PUFA). As there are multiple possibilities for the position of double bonds, it is important to identify the exact position. The omega (ω) system, also sometimes given the abbreviation 'n' indicates how many carbons there are from the methyl end to the first double bond (McClements & Decker 2017). Hence, omega-3 has its first double bond three carbons from the methyl end.

Omega-3 has similar biological activity in their ability to decrease triacylglycerol's levels. Therefore, the omega system helps to categorize the fatty acids according to their bioactivity and biosynthetic origin (ibid).

3.2 Biosynthesis of PUFA

There are many different types of PUFAs the omega-6 and omega-3 families are the two principal ones. The most common members of each family are linoleic acid (LA; 18:2n-6) and α- linoleic acid (ALA; 18:3n-3), which cannot be synthesised by the human body. However, they can metabolise these PUFAs to other fatty acids, (Fig 1) by insertion of additional double bonds and by elongation of the acyl chain. With the help of enzymes and a series of reaction LA can be converted into Arachidonic acid (AA; 20:4n-6), the latter and ALA act as substrate for synthesis of Eicosapentaenoic acid (EPA; 20:5n-3). EPA can be further metabolised to Docosapentaenoic acid (22:5n-3) and Docosahexaenoic acid (DHA; 22:6n-3) (Calder 2008).

The production of DHA and EPA from ALA is possible through elongation and desaturation, however it is limited and slow. The enzyme responsible for synthesizing from ALA and LA is △-6 desaturase. The rate of its activity is affected by aging, stress, lack of nutrition, some types of infections, saturated, trans-fats and lifestyle choices such as caffeine and alcohol. Therefore, the reaction is very slow, the EPA converted from ALA is diminutive and the DHA even less (Lane *et al.* 2014).

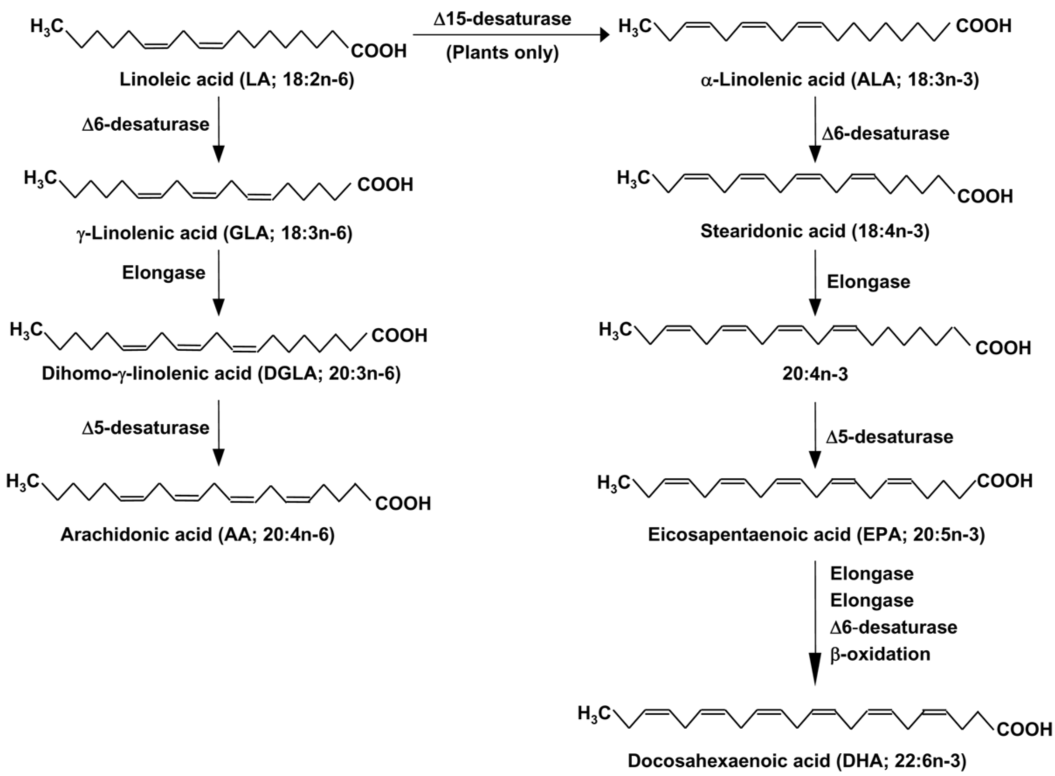

Figure 1. Biosynthesis of PUFAs (Calder 2008)

Enzymes often recognize the fatty acids from their free methyl end when it is esterified to glycerol (McClements & Decker 2017).

3.3 EPA & DHA

Important to understand is that there are different types of omega-3s, the three main ones are Alpha-linolenic acid (ALA), Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) (Topuz, 2016). DHA and EPA are essential for human health as they support healthy heart, blood circulation and immune response (Winwood 2013).

Eicosapentaenoic acid (EPA) uses the prefix 'eicosa' due to its 20 C atoms and 'penta' significates that it contain 5 cis-double bonds. Docosahexaenoic acid (DHA) gets its name from the 22 C atoms, 'docosa' and 'hexaenoic' because it contains 6 cis-double bonds (Topuz 2016). (Fig 2)

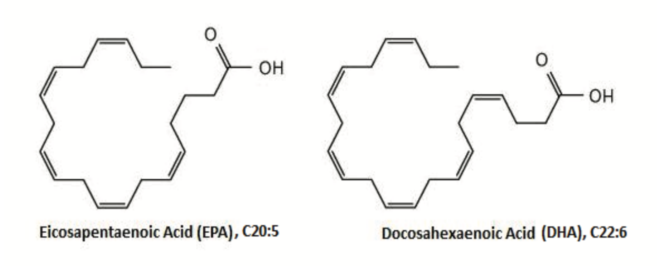

Figure 2. Structure of EPA and DHA (Topuz, 2016)

Already for years studies have been showing that EPA and DHA have different effects and potencies. EPA seems to have a stronger effect on reducing inflammation and depression, whereas DHA seem to have stronger effect on modifying blood lipid profiles (Topuz 2016). As well as specializes in the areas of the eye, heart and brain (Winwood 2013).

When researching DHA and EPA metabolism, measurements of DHA tend to be greater than EPA, indicating that there are different pathways that metabolise each FA. Studies have also shown that DHA might be blocking the effects of EPA (Kagan *et al.* 2013).

3.4 Functions

PUFAs play an important role in cells, they maintain membrane fluidity as well as regulating the correct environment for protein function and controlling cell signalling. Adjusting cellular function and gene expression is another of the PUFAs many functions. EPA are involved in regulation of many cell and tissue responses (Calder 2008).

4. Sources of Omega-3

4.1 Fish oil

Fish oil varies considerably depending on the diet of the fish and its habitat. This will affect the quality and amounts of fatty acids it will produce. They can range between C12 to C24, this varies not only between species but also within. The most regarded fish oil are the ones with LC-PUFA, which have an abundance of EPA and DHA. The source of these oils is fatty fishes, one popular one with high amounts of the desired omega-3 is the Peruvian anchovy (De Silva et al. 2010).

Many fish have lost the ability to desaturate and elongate the PUFAs, LA and ALA into EPA and DHA (De Silva *et al.* 2010). Therefore, they are very dependent on the LC-PUFA they get from the feed they eat (Calder 2008).

Fish oil is mainly taken from fish with rich triglyceride (TAG), as it is extremely good in storing fat and it can contain stored fat up to 90% of the total fatty acid composition. (De Silva *et al.* 2010). In fish oil the omega-3 are mainly conjugated to a TAG, whereas in krill oil the FA are conjugated to phospholipids (PhLs) (Kagan *et al.* 2013).

There are naturally occurring chemicals in fish oils, depending on the source, however there is a noticeable problem with persistent organic pollutants (POP) which include organochlorine pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls (PCB), polybrominated diphenyl ethers and dioxins. PCB is known to increase possibilities to get cancer and prevents viability in humans. POP bioaccumulates in the lipid component of fish tissue and perseveres the fish oil extraction (De Silva *et al.* 2010).

4.2 Algae

Since billions of years micro-algae evolved pathways to convert ALA to DHA using enzyme △4-desaturase. These phytoplankton remain the primary source of

LC-PUFAs (Winwood 2013). Algae provide foundation of the oceanic food chain, as they are the primary producers in the ocean's ecosystem. Algae synthesize omega-3 which are further eaten by small fish such as krill which are further consumed by carnivore fish such as tuna and salmon. Micro-algae include all microscopic algae whether they are unicellular or filamentous (Lenihan-Geels *et al.* 2013).

Most algae are autotrophic therefore they require light for growth, heterotrophic algae, which utilise organic compound as nutrients are also available. However, some autotrophic algae can adapt to heterotrophic conditions, they metabolise glucose to produce energy when light is not available (Winwood 2013).

Lipids produced by algae come up to between 1-70% of the algaes cell weight, under optimal conditions this can be increased to up to 90%. The interesting ones are the EPA and DHA (ibid). These PUFAs are found in phospholipids in krill and as glycolipids in algae, the latter one seems to be more effective in human diets (Kagan *et al.* 2013).

Microalgae is not just an interesting alternative source of omega-3, they also contain many other valuable compounds to human health, such as sterols and pigments (Haimeur *et al.* 2016).

4.3 Suitable microalgae

It is important to find the suitable algae for extraction of omega-3, as there are such a huge variety of species. The most popular micro-algae for commercial use are from the families *Thraustochytriacea* and *Crythecodiniacea,* the latter one includes the genus *Crythecodinium*, *Schizochytrium* and *Ulkenia* are genus of the first mentioned family. The advantage of this family is that they are heterotrophs (Winwood 2013).

Crypthecodinium cohnii is special as it only produces DHA and can fix carbon dioxide which is both cost efficient and sustainable (Topuz, 2016). *Thraustochytrium* spp. has also been investigated to produce high DHA of up to 35% total FA (Lenihan-Geels *et. Al* 2013). On the other hand *Schizochytrium* produce both EPA and DHA (*Kaga et. Al* 2013). Two other species with the same characteristics are *Bacillariophyceae* (diatoms) and *Chrysophyceae.* (Topuz, 2016). One authorized, following EU-regulation 258/97, as a novel food supplement rich in omega-3, is *Odontella aurita* (OA), which is a diatom and rich in EPA (Haimeur *et al.* 2016).

Autotroph algae usually contain higher levels of EPA whereas it is more common to use heterotrophic microalgae for DHA production (Topuz 2016). However heterotrophic processes usually have higher production yield compared to photoautotrophic processes (Gu *et al.* 2021).

Many of the algae do not only have EPA or/and DHA but also several other bioactive compounds, such as pigment, fibers and phytosterols which are beneficial effects on human health (Haimeur *et al.* 2016).

Having said this, the mentioned organism presents a promising and non-pollutant resource of omega-3 and much more, as an alternative to fish oil (Topuz 2016).

4.4 Production

The production of algae is considered sustainable as it does not require large arable land to grow (Lopes *et al.* 2017). Open culture productions have low costs owing to the fact that only sun light is needed to accumulate FA, which is environmental friendly, however, open system are susceptible to contaminations. The exposure to seasonal temperatures also affects the growth rate and composition (Gu *et al.* 2021). The prices of controlled algae fermentation, which are used for the FDA approved oils are expensive, slow and often produces low yields (Lopes *et al.* 2017). When using autotrophic algae, the light creates limitation to the size of the production (Winwood 2013). Appropriate conditions for growth of algae are essential, as studies show that low nitrogen levels with high supply of monosodium glutamate and yeast extract resulted in best growth and synthesis of fatty acids (Lenihan-Geels *et al.* 2013).

Today there have been big developments on how to optimize growth which leads to the production of pure EPA and DHA oils containing up to 30 – 40% of target FA. Many micro-algae oil produced under controlled conditions have today also been approved by FDA as they have demonstrated safety nutritional profiles (Lopes *et al.* 2017).

The way fish is depleted from the sea is not sustainable, it’s impossible to keep up with the human fish consumption, the use for feed as well as for fish oil. There are already noticeable environmental effects from the overfishing and inefficiency of the use of fish. The quality of the omega-3 relies on the diet of the animal, therefore farmed fish is not the best source, as it often is fed products which are not natural fish feed (Winwood 2013).

Oily fish is what is used for omega-3 production, such as tuna, salmon and mackerel. One medium size meal with one of these fish will provide between 1.5 to 3.5 g of the long chain n-3 PUFAs like EPA and DHA, which is the equivalent of 1g fish oil capsule providing 300mg of FA (Calder 2008). In comparison from 100kg fresh fish one will get around 5kg of fish oil.

However, one of the big problems with omega-3 from fish oil is that only 5% of the world fish oil production extracts n-3 for human consumption, most of the fish oil goes to fish farming (Ciriminna *et al.* 2016). To handle the increasing demand for omega-3 supplements and the decreasing availability of fish, there needs to be a more efficient way to use the seafood 'waste' (Ciriminna *et al.* 2016).

4.4.1 Extraction

Extraction of the omega-3 from micro-algae, can be achieved through different methods. The initial step is to rupture the cell wall followed by extraction with solvent, mostly used is hexane. During the rupturing of the cell wall, LC-PUFAS are immediately exposed to potential oxidation, which makes this process complicated. Once there is a reaction with oxidised radicals, there is an unstoppable chain of reactions which makes the oil rancid with strong odours unsuitable for consumption. Therefore, it is extremely important to avoid all source of material that can initiate this type of reactions. This also applies for the fish oil extraction.
Solventless extraction processes have been developed, however the most common way is by using hexane. The crude algal oil is kept cold usually using nitrogen until the next step when refining is done. The leftover biomass contains some omega-3 and is therefore used in animal feed (Winwood 2013).

Once caught, the fish are cooked and pressed on the ship, the water-oil mixture is separated from the protein by filtration which is stored, becoming fishmeal. Omega-3 extraction process starts at industrial sites, where the water is removed from the oil with 3-phase centrifuge (Ciriminna *et al.* 2016).
Conventional omega-3 production from fish is based on molecular distillation. In this process the one long-chain omega-3 PUFA which is bound to one glycerol molecule needs to be converted into ethyl esters using ethanol followed by distillation under ultralow pressure under high temperatures. In 2010 when it was discovered that omega-3 in triglyceride form is absorbed better by humans, a new process where the omega-3 ethyl esters are transformed back into TGA form was brought in. Today, several manufacturers use this method for their supplements (Ciriminna *et al.* 2016).

Recent technologies have discovered new methods for oil extraction, to avoid chemical products and high temperatures. These can involve supercritical fluid technology, together with membrane and enzymatic processes. (Topuz 2016)

4.4.2 Refining

Once the oil has been extracted there are many components that need to be removed to acquire the desired colour, flavour and shelf-life. During extraction one contains the crude oil and fats, there are also free fatty acids, phospholipids, lipid-soluble off flavours and carotenoids, as well as proteins and carbohydrate (McClement& Decker 2017). Traditional refinement for both fish and algae oil, is usually done by chemical methods such as:
Chemical refining and caustic soda for removing free fatty acids. Physical refining uses high temperature therefore less suitable. Degumming is used for neutralisation and removing phosphatides and sterols. Bleaching, by using clay or carbon, removes colour pigments, oxidation products and traces of metal. Last remaining oil components that may affect odour or taste which might have been produced in the refining process, are removed by high pressure steam, followed by cooling down. Finally, oil gets dewaxed to improve clarity and antioxidants are added for more stability during storage (Winwood 2013).

Once fish oil has been refined it contains about 30% omega-3 (18% EPA, 12% DHA) and the remining 70% is a mixture of components including omega-6, cholesterol, saturated fatty acids, oxidation products and contaminants (Ciriminna 2017). The composition of algae oil depends on which algae is being used, as some have both EPA and DHA, others only have one of the. However, taking *Nannochloropsis* as an example the composition is 20 – 30% omega-3 (only EPA) and the remaining 70% contains omega-7 lipids, proteins and carbohydrates. (USPTO 2014)

5. Health benefits

5.1 Inflammatory diseases

Omega-3 PUFAs have shown positive effects on the inflammatory pathway resulting in beneficial outcomes in inflammatory bowel disease IBD, arthritis and cardiovascular disease as well as some cancers (Lenihan-Geels *et al.* 2013).

There is strong evidence that chronic inflammations have a direct link to some of the most common health problems worldwide such as cancer. Studies have shown a correlation between the survival rate of colorectal cancer patients and the amount of particularly cytokines found. Another parallel discovery is with inflammatory markers and size of prostatic tissue and its progression (ibid). It is also known that n-3 PUFAs from fish oil limit tumor cell proliferation, increase apoptotic potential along the crypt axis, promote cell differentiation, and possibly limit angio- genesis (Roynette *et al.* 2004).

Stearidonic acid (SDA) and EPA have shown to reduce the tumor necrosis factor-alpha (TNF-alpha), a well-known pro-inflammatory-cytokine involved in cancer. Their effects are dependent on sex, race and genetics of the patients and type of cancers. However, a diet which is unproportionally in n-3 and n-6 could increase cancer risks. This is due to the two enzymes COX and LOX compete against each other which affects the synthesis of EPA (Lenihan-Geels *et al.* 2013).

The anti-inflammatory properties have been success in treating inflammatory diseases; eczema, psoriasis as well as cardiovascular diseases (Lane *et al.* 2014).

5.2 Cardiovascular disease (CVD)

The beneficial effect of n-3 from micro-algae in human health, in particular preventing CVD, has been identified in several studies (Haimeur *et al.* 2016). Other benefits such as lowering blood pressure and heart rate; reducing serum triglycerides, inflammation and arrhythmias have also been discovered (Lane *et al.* 2014).

The European Food Safety Agency (EFSA) recommends a daily intake of 250 mg/d of EPA and DHA for adults, because such an intake is linked to lower incidences of CVD (EFSA 2010).

5.3 Neurological diseases

A deficiency in certain essential fatty acids, particularly the omega-3 DHA creates neuronal functions to be impaired affecting the neurotransmission actions. Therefore, it is recognized that DHA and EPA play a protective role in brain development and in retarding neurological pathologies such as Alzheimer, Parkinson’s and Huntington’s disease MS, Schizophrenia, cognitive decline, brain ageing, major depression, acute stress and anxiety. (Lopes *et al.* 2017)

Studies have also shown that a low EPA creates depressive disorders in patients, resulting in lower survival rate (Lenihan-Geels *et al.* 2013).

6. Comparative studies – Fish oil vs Algae

Several studies have been made to investigate the difference between algae oil and fish or krill oil, and its effects human nutrition. Some are mentioned here below:

A study done with young males who were fed a fatty meal followed by either krill oil containing EPA and DHA, or algae EPA oil showed that consuming this specific EPA oil, *Nannochloropsis oculate,* was very effective in enhancing EPA levels in human nutrition (Kagan *et al.* 2013).
You could see an increase of EPA with both krill and algae oil when consumed. However, there was a significant maximum concentration of EPA, found in plasma, from algae oil supplementation at various points of the day. One explanation could be that DHA limits the incorporation of EPA into plasma lipids, another could be that PUFAs from algae oil are found in glycolipids which is more effective in human diets (Kagan *et al.* 2013).

Heimur *et. al* (2016) conducted a study to compare the effects of oil from algae *O. aurita* (OA) and fish oil on some risk factors involved in CVD, including hyperlipidemia, insulin-resistance, diabetes, thrombosis and oxidative stress. Previous research has reported that supplementing n-3 PUFA has been beneficial in high fat diets with 40% saturated fats. The outcome showed OA supplementation enhanced insulin sensitivity and decreased both TAG and cholesterol levels, by reducing lipid accumulation in the liver. This indicated that OA could regulate lipid metabolism in a similar way to fish oil (Heimur *et al.* 2016).

A comparative study testing the proliferation and viability of colonic cells, Caco-2, was done using algae and fish oil. Algae oils used, derived from heterotrophic micro-algae *Crypthecodinium cohnii* and *Schizochytrium sp,* rich in DHA, together with *Nitzschia laevis* with high EPA levels. Fish oil derived from menhaden, a fish from the herring family. Despite the different PUFAs content in the fish and algal oil, the results showed a similar effect on cancer cells in vitro. This was attained in the mitochondrial activity and in the quantification of proliferation assays (van Beelen *et al.* 2007).

7. Discussion

There are both advantages and disadvantages with both algae and fish oil. Let's summarise; fish production is inexpensive compared to algae, which is costly and a slow process. Fish contains both EPA and DHA, whereas algae mostly contains one of the two FA. However, the oils usually contain different species to reach the desired quantities of EPA and DHA. The EPA and DHA ratio is usually more consistent in algae, compared to fish which is dependent on the species and what it eats. These interesting omega-3 from algae are expensive on a weight-to-weight basis (Winwood 2013). Although this source does guarantee contaminant free FA. While there are differences, the importance lies in the similarities of the effects in human nutrition.

DHA and EPA are essential for human nutrition, as they have health benefits which affect some of the largest public health problems worldwide; heart and inflammatory diseases, as well as mental illnesses. Having looked at several studies that imply that LC omega-3, specifically DHA and EPA, help prevent the health issues mentioned, one can be assure that this is an important supplement for humans. As fish oil is not a sustainable way forward, the discovery of algae oil is a promising alternative.

Several studies done by Arterburn *et al.* (2007), Sanders *et al.* (2006) and Geppert *et al.* (2006), using small dosages of algal oil containing DHA, noted significant increase levels in the plasma. This shows algae oil as a very good source for vegetarians, which in their studies could not achieve these levels of DHA by other LC-PUFA sources such as flaxseed oil, walnut and other oils. (Lane *et al.* 2014) Another interesting finding was that DHA could be retro conversed to EPA, suggesting DHA could be a source of EPA (Conquer & Holub 1997).

Fish oil contains both EPA and DHA, because the fish gets it from the feed. As it cannot synthesize these FA it will feed from krill or other smaller fish which live on micro-algae. In this way the fish will contain both EPA and DHA (Winwood 2013). Algae which are the primary source of the interesting FA, will often only contain one or the other. This can be useful if you just want to increase the levels of one of the omega-3 FA. For instance, there are indications that EPA can reduce the TNF-alpha involved in cancer, therefore using an algae with just that FA could be an advantage (Lenihan-Geels *et al.* 2013). Specifically with EPA, this could be helpful as there are some suggestions that DHA could limit the incorporation of EPA (Kagan *et al.* 2013). However, there are continuous developments and new processes discovered, which have led to existing algae supplement with both DHA and EPA (Winwood 2013).

The comparative studies reviewed in this project have shown that algal oil is a favourable alternative to fish oil. In some cases, it resulted in being an even better choice, where algae *O. aurita* (OA) oil was more effective in reducing triaglycerol levels and in preventing high-fat diet induced steatosis (an abnormal retention of lipids within a cell or organ) (Heimur *et a.l* 2016). On the other hand, this study also showed that fish oil was a better source for EPA uptake in the plasma phospholipids. However, the conclusion from trying both OA algae and fish oil rats fed high-fat diets was that OA algae produced similar biological effects and is favourable for preventing CVD risk factors (Heimur *et al.* 2016). Another fact that makes algae a better source is the fact that it is not polluted, cultivated in closed facilities assures that the EPA and DHA are clean sources.

Algae seems to be an equivalent source of omega-3 to fish oil. It is a more sustainable source by not using up big areas of arable land, it is very efficient in fixating carbon dioxide and it’s a direct source of EPA and DHA. In that case, why has it not been grown more commercially as a supplement? Although it has become more popular, fish oil dictates the market. One of the obvious reasons is there is not enough research in the field. Identifying suitable strains is one of the keys for success (Gu *et al.* 2021). Not only is it important for the certainty that there are not any impurities or pollutants that could be damaging, but also for high productivity and lowering the costs. Nevertheless, achieving optimal ingestion and digestibility is just as important (Lenihan-Geels *et al.* 2013).

There are some great interests in bioengineering for improving algae omega-3. Therefore, this marine source has big potentials for the future (Topaz 2016). Especially as the demand of omega-3 supplement is growing and the fish industry is turning into a hazard for the environment.

References

Arterburn, L. M., Oken, H. A., Hoffman, J. P., Bailey-Hall, E., Chung, G., Rom, D., Hamersley, J. and McCarthy, D. (2007). Bioequivalence of docosahex- aenoic acid from different algal oils in capsules and in a DHA-fortified food. *Lipids.* 42, 1011-1024. Doi: 10.1007/s11745-007-3098-5

Calder, P.C. (2008). Polyunsaturated fatty acids, inflammatory process and inflammatory bowel disease. *Molecular Nutrition & Food research*. Volume 52(8), 885-897. https://doi.org/10.1002/mnfr.200700289

Ciriminna, R., Meneguzzo, F., Delisi, R. & Pagliaro, M. (2017) Enhancing and improving the extraction of omega-3 from fish oil. *Sustainable Chemistry and Pharmacy*. Volume (5), 54-59. http://dx.doi.org/10.1016/j.scp.2017.03.001

Conquer, J. A. and Holub, B. J. (1997). Dietary docosahexaenoic acid as a source of eicosapentaenoic acid in vegetarians and omnivores. *Lipids*. Volume (32), 342-345.

De Silva, S.S, Francis, D.S & Tacon, A.G.J. (2010). Fish oil in aquaculture. I: Turchini, G.M., Ng, W.K. & Redford Tocher, D. (red) *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*. Boca Raton: Taylor & Francis Group. 1-20. https://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=665640

EFSA. (2010). *EFSA sets European dietary reference values for nutrient intake.* https://www.efsa.europa.eu/en/press/news/nda100326 [2022-05-23]

Gu, W., Kavanagh, J.M. & McClure, D.D (2021). Towards a sustainable supply of omega-3 fatty acids/ Screening microalgae for scalable production of eicosapentaenoic acid (EPA). *Algal Research*. Volume (61). https://doi.org/10.1016/j.algal.2021.102564

Harris, W.S., Calder, P.C., Mozaffarian, D. & Serhan, C.N (2021). Bang and Dyerberg's omega-3 discovery turns fifty. *Nature Food*. Volume (2), 303-305. https://doi.org/10.1038/s43016-021-00289-7

Geppert, J., Kraft, V., Demmelmair, H. and Koletko, B. (2006). Microalgal sources of docosahexaenoic acid decreases plasma triacylglycerol in nor- molipidaemic vegetarians: A randomised trial. *British Journal of Nutrition*. Volume (95): 779-786. Doi: 10.1079/bjn20051720

Haimeur, A., Mimouni, V., Ulmann, L., Martineau, A-S., Messaouri, H., Pineau-Vincent, F., Temblin, G. & Meskini, N. (2016). Fish Oil and Microalga Omega.3 as Dietary Supplements: A Comperative Study on Cardiovascular Risk Factors in High-Fat Fed Rats. *Lipids*. Volume (51), 1037-1049. Doi 10.1007/s11745-016-4177-2

Kagan, M.L, West, A.L., Zante, C. & Calder, P.C. (2013). Acute appearance of fatty acids in human plasma – a comparative study between polar-lipid rich oil from the microalgae Nannochloropsis oculata and krill oil in healthy young males. *Lipids in Health and Disease*. Volume (12), 102 (2013). Doi: https://doi.org/10.1186/1476-511X-12-102

Lane, K., Derbyshire, E., Li, W. & Brennan, C. (2014) Bioavailability and Potential Uses of Vegetarian Sources of Omega-3 Fatty Acids: A review of the Literature. *Food and Science and Nutrition.* Volume (54). 572-579. https://doi.org/10.1080/10408398.2011.596292

Lenihan-Geels, G., Bishop, KS. & Ferguson, L.R. (2013). Alternative Sources of Omega-3 Fats: Can We Find a Sustainable Substitute for Fish? *Nutrients*. Volume (Number), 1301-1315. doi: 10.3390/nu5041301

Lopes, P.A, Bandarra, N.M, Martins, S.V., Martinho, J., Alfaia, C.M, Madeira, M.S, Cardoso, C., Alfonso, C., Paulo, M.C, Pinto, R.M, Guil-Guerrero, J.L and Prates, .A.M (2017). Markers of neuroprotection of combined EPA and DHA provided by fish oil are higher than those of EPA (Nannochloropsis) and DHA (Schizochytrium) from microalgae oils in Wistar rats. *Nutrition & Metabolism*. Volume (14),62. DOI: 10.1186//s12986-017-0218-y

McClements, D.J & Decker, E.A. (2017). Lipids. I: Damodaran, S. & Parkin, K.L (ed). *Fennema's Food Chemistry*. Ed. 5th, Florida: CRC Press. 171-234

Sanders, T. A. B., Gleason, K., Griffin, B. and Miller, G. J. (2006). Influence of an algal triacylglycerol containing docosahexaenoic acid (22:6n-3) and docosapentaenoic acid (22:5n-6) on cardiovascular risk factors in health men and women. *British Journal of Nutrition.* Volume (95), 525-531. Doi: https://doi.org/10.1079/BJN20051658

Schuchardt, J.P., Schneide, I., Meyer, H., Neubronner, J., von Schacky, C. & Hahn, A. (2011). Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acids formulations – a comparative bioavailability study of fish oil vs. krill oil. *Lipids in Health and Disease*. Volume (10), 145 (2011). Doi: https://doi.org/10.1186/1476-511X-10-145

Topuz, O.K. (2016). Algal oil: a novel source of omega-3 fatty acids for human nutrition. *Scientific Bulletin. Series F. Biotechnologies*. Volume 20, 178-183. http://biotechnologyjournal.usamv.ro/pdf/2016/Art30.

United States Patent and Trademark office (USPTO) (2014). *Algal oil composition*. (US-2014: 0275483-A1). California: Patent application publication.

Van Beelen, V.A., Roeleveld, J., Mooibroek, H., Sijtsma, L., Bino, R.J., Bosch, D., Rietjens, I.M.C.M & Alink, G.M. (2007). A comparative study on the effect of algal and fish oil on viability and cell proliferation of Caco-2 cells. *Food and Chemical Toxicology.* Volume (45), 716-724. Doi:10.1016/j.fct.2006.10.017

Winwood, R.J (2013). Algal oil as a source of omega-3 fatty acids. I: Jacobsen, C. & Nielsen, N.S (red). *Food Enrichment with Omega-3 Fatty Acids*. Woodhead UK: Publishing Limited. 389-404. Doi: 10.1533/9780857098863.

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