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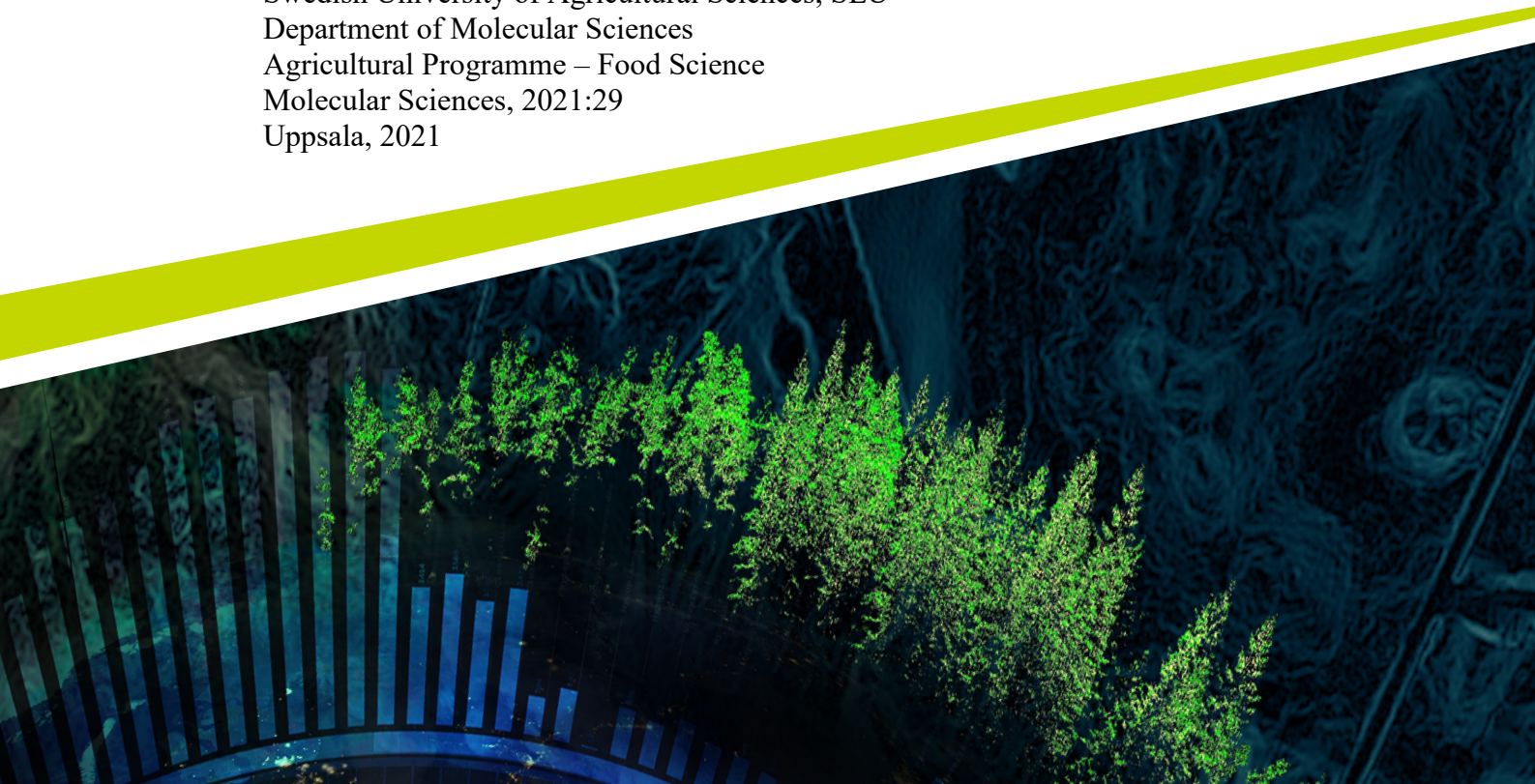

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Abstract

Microalgae are a promising source of high value compounds for the food industry with both nutritional and health-beneficial qualities with potential to meet the dietary demand of the growing population. Microalgae are highly adaptive towards environmental stresses which is used as important tool for manipulation of the biomass for enhancement of metabolites.

Diverse species have been exploited so far and a handful are currently cultivated in large scale. However, one of the main challenges is to make them economically feasible for the production of industrially useful biomolecules. Some of the compounds in microalgae have low bioavailability due to low digestibility, therefore efficient technologies for extraction of these compounds are often needed. Recent technological advances in this field have been made to establish more scalable and sustainable methods to avoid the toxic solvents which often are involved and acquiring compounds with high purity.

Important microalgae species for human nutrition include *Chlorella* and *Arthrospira* spp. which show higher protein contents compared to more common protein sources. Also, *Crypthecodinium cohnii* and *Schizochytrium* sp. have become important due to their capacity of producing high amounts of the omega-3 fatty acid docosahexaenoic acid (DHA). There is an established market for microalgal pigments with an especially high demand for β-carotene. Edible microalgae have been for a long time and still are an accepted part of the common diet in many Asian countries, while the Western culture, it still has a long way to go. The possibility of using microalgae and algae-derived compounds for functional foods have however gained increased interest in recent years and with the increasing development of algae-based products on the market, eventually, they might be a regular part of many people's diet.

Keywords: Microalgae, food, growth conditions, biochemical compounds, extraction

Sammanfattning

Mikroalger är en lovande källa till högkvalitativa biomolekyler med nytta för livsmedelsindustrin med både näringsrika och hälsofördelaktiga egenskaper, och potential att tillgodose den växande befolkningens kostbehov. Mikroalger är otroligt anpassningsbara mot olika typer av abiotiska stressfaktorer vilket används som verktyg för att manipulera biomassan och förbättra näringsinnehållet.

Idag nyttjas ett flertal arter och en handfull av dessa odlas industriellt i stor skala. En av de största utmaningarna är dock att göra dem ekonomiskt hållbara för produktion av industriellt användbara biomolekyler. Vissa föreningar i mikroalger har låg biotillgänglighet på grund av dålig smältningsgrad vilket medför att man behöver använda olika tekniker för att extrahering av ämnen. Nya tekniska framsteg inom detta område har gjorts för att skapa mer expanderbara och hållbara metoder för att undvika de giftiga lösningsmedlen som ofta används idag för att kunna erhålla rena fraktioner.

Två betydelsefulla mikroalger som används för mänsklig konsumtion är *Chlorella* och *Arthrospira* spp. som har ett högt proteininnehåll jämfört med flera vanligare proteinkällor. *Crypthecodinium cohnii* och *Schizochytrium* sp. har också blivit populära på grund av sin förmåga att producera stora mängder av omega-3 fettsyran dokosahexaensyra (DHA). Det finns en väl etablerad marknad för pigment från mikroalger med särskilt hög efterfrågan på β-karoten. Mikroalger som födokälla har länge varit och utgör än idag en del av kosten i många asiatiska länder, medan den västerländska kulturen fortfarande ligger långt efter.

Möjligheten att använda mikroalger samt erhållna extraherade ämnen för funktionella livsmedel har emellertid fått ökat intresse de senaste åren och med den ökande utvecklingen av algbaserade produkter på marknaden kan de i framtiden bli en basföda.

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Abbreviations

ALA	α-linolenic acid
BM	Bead milling
DHA	Docosahexaenoic acid
EAA	Essential amino acids
EPA	Eicosapentaenoic acid
HPH	High pressure homogenizer
HSH	High speed homogenizer
PBR	Photobioreactor
PUFA	Polyunsaturated fatty acids
UNS	Ultrasonication

1. Introduction

Microalgae are a large group of unicellular organisms, constituting the basis of the aquatic food chain. They comprise a highly diverse group of prokaryotes and eukaryotes (Chacón-Lee & González-Mariño 2010), growing in a wide range of aquatic environments such as rivers, lakes, ponds, oceans and wastewaters (Khan et al. 2018). Their photosynthetic machinery is similar to that of terrestrial plants but they are in general more efficient in converting solar energy into high amounts of biomass (Chacón-Lee & González-Mariño 2010).

The number of microalgae species have not been established, but is has been estimated that 200,000 to 800,000 species exist, while only a small amount of these are cultivated at larger scale (Koyande et al. 2019). The research of microalgae for industrial applications is extensive and they find uses in the production of food, feed, biofuels and many more (Camacho et al. 2019).

In 2012, the European Union came up with a strategy called “Innovating for Sustainable Growth: A Bioeconomy for Europe”, as an approach to increase sustainable productions and to decrease negative impacts on the environment. Since this, the EU is granting funding's to improve the use of innovative and sustainable renewable resources as well as at the same time try to satisfy the demand for food, energy and other products. Aquatic resources are raising large possibilities in light of the EU bioeconomy, and microalgae are especially attractive as sources of a broad range of valuable molecules for the food and nutrition sector (Vigani et al. 2015).

Microalgae have been used as food for thousands of years, however, the commercial cultivation of microalgae is only a few decades old (Spolaore et al. 2006). Cultivation is commonly carried out in open pond systems or in photobioreactors (PBRs) (Panahi et al. 2019) and the chemical composition of microalgae varies greatly with species and cultivation conditions. However, the general composition is 40-70% proteins, 12-30% carbohydrates, 4-20% lipids, 8–14% carotenoids, and varying amounts of minerals and vitamins (Garcia-Vaquero 2021).

Although much research about microalgae-based foods are promising and a variety of products are existing or being commercialized the market is still limited. Regardless of this, there is an increasing interest and demand for microalgae in foods, pharmaceuticals and in many other fields.

The purpose of this literature study is to investigate important features of edible microalgae: nutritional value, applications, cultivation and extraction methods for obtaining high value compounds.

1.2 Method

This literature study was conducted by collecting and assembling research literature about the subject. The information has been obtained from the search engines such as *Primo, PubMed, NCBI, Scopus and MDPI.* Following keywords were used in different combinations: *Microalgae, EPA, DHA, fatty acids, lipids, carotenoids, proteins, peptides, carbohydrates, Dunaliella, food, cultivation, PBR, open pond, Arhrospira, Chlorella, Schizochytrium, Dunaliella, pigments, extraction methods, saccharides, milking, bead milling, mechanical, chemical, solvents, microwave, homogenization.*

2. Result and discussion

2.1. Microalgae as a source of lipids

Lipids are one of the essential macronutrients, accounting for around 30% of the total energy intake in a healthy balanced diet. Similarly, to plants, the two main lipid groups in microalgae are (i) polar lipids, such as various phospholipids and galactolipids and (ii) non-polar lipids (neutral lipids) which include acylglycerols, free fatty acids and carotenoids. Polar lipids are structural components of cell membranes and the neutral lipids function as energy storage in microalgae (Mimouni et al. 2018; Barkia et al. 2019). Microalgae have been identified as producers of large amounts of lipids (30-50%), depending on species but also on growth phase and cultivation conditions (Garcia-Vaquero 2021). However, lipid values ranging from 1-70% and up to 90% of dry weight have been reported (Spolaore et al. 2006). Generally, during the exponential growth phase of microalgae, most species have a high content of polar lipids, and under stress conditions such as nutrient limitation they often accumulate neutral triacylglycerols (TAGs).

Considering fatty acids, microalgae generally contain a mixture of C16 and C18 saturated (SFAs) and monounsaturated fatty acids (MUFAs), as well as polyunsaturated (PUFAs), including essential omega fatty acids (Barkia et al. 2019). The neutral TAGs mostly contain SFAs and MUFAs, whereas PUFAs are mainly associated with the polar lipids (Shen et al. 2016).

Health benefits of fats are correlated with the composition of the lipids, and consumption of PUFAs have been linked to the prevention of diseases such as cardiovascular diseases (Garcia-Vaquero 2021).

2.1.1. Microalgal PUFAs

Several microalgal species are valuable sources of essential fatty acids which cannot be synthesized by humans and therefore must be obtained through the diet. This is the case for the omega-3 and omega-6 PUFAs linoleic acid (LA, 18:2:n-6), n-3, α-linolenic acid (ALA, 18:3:n-3) and long chained PUFAs such as, arachidonic acid (ARA, 20:4 n-6), eicosapentaenoic acid (EPA, 20:5:n-3),
and docosahexaenoic acid (DHA, 22:6:n-3) (Remize et al. 2021). For a long time, microalgae have especially been attracted as a source of ALA, EPA and DHA which have gained attention due to their obvious health benefits for humans (Caporgno & Mathys 2018).

To date, EPA and DHA for human nutrition are mainly obtained from aquatic organisms, such as fatty fish, while the main sources of ALA are vegetable oils such as canola, soybean and flaxseed oil, but also walnuts, chia and some green leafy vegetables. ALA is the precursor of EPA and DHA and can be converted into these in the human body. However, in humans as well as other mammals, the process of synthesizing EPA and DHA from ALA is very inefficient (Caporgno & Mathys 2018; Peltomaa et al 2018; Rajaram 2014). Microalgae at the bottom of the food chain, are the initial producers of EPA and DHA that eventually are accumulated through the trophic levels. Changes in lipid composition of microalgae are carried through the food chain and affects the growth and composition of zooplankton, crustacean larvae, mollusks and some fish, which in turn affect accumulation of DHA and EPA in humans and other higher organisms (Adarme-Vega et al. 2012).

Many microalgae species naturally produce high contents of DHA and EPA. To date, most microalgae used for the production of DHA rich oils are marine algae, part of the families *Thraustochytriacea* such as *Schizochytrium* sp. and *Crypthecodiniaceae* e.g., *Crypthecodinium* sp. They are both heterotrophic microalgae which implies that they can grow in the dark and require an organic carbon source. They exhibit a high lipid content of up to 50-77% in dry weight and their fatty acid profile typically show high contents of DHA and a range of other fatty acids (Lopes da Silva et al. 2019). *Phaeodactylum tricornutum* is a marine autotrophic diatom known for its high production of EPA (Domergue et al. 2003). *Crypthecodinium cohnii* and *Schizochytrium* sp. show DHA contents of 50-60% and 35-40% of total fatty acids respectively and *Phaeodactylum tricornutum* has been reported to accumulate approximately 30% EPA, but only small traces of DHA (table 1) (Domergue et al. 2003).

Table 1. Listed content of chosen fatty acids in microalgae species (% of total fatty acids).

Fatty acid	Common name	*Crypthecodinium cohnii*	*Schizochytrium* sp.	*Phaeodactylum tricornutum*
14:0	Myristic acid	14-16	9-15	5.5
16:0	Palmitic acid	10-14	24-28	16.6
16:1	Palmitoleic acid	2.0-3.0	0.2-0.5	25.9
18:0	Stearic acid	0.0-0.3	0.5-0.7	n.d.
18:1	Oleic acid	9.0-10	n.d.	2.4
18:3 (n-3)	α-linolenic acid (ALA)	n.d.	n.d.	< 1.0
20:5 (n-3)	Eicosapentaenoic acid (EPA)	n.d.	n.d.	30.2
22:6 (n-3)	Docosahexaenoic acid (DHA)	50-60	35-40	n.d.

No data: n.d.
Table references: (Domergue et al. 2003; Ratledge et al. 2010).

The bioavailability of both DHA and EPA in algal oils are high for humans, ranging from 50-100 % (Wells et al. 2016).
Countless trials support the health benefits of both DHA and EPA (Allaire et al. 2016; Miller et al. 2014; Li et al. 2014). They are thought to have powerful anti-inflammatory affects, to play a part in oxidative stress and to improve cell functions. DHA and EPA have, for instance, been associated with normal fetal development, improved cardiovascular function and prevention of Alzheimer's disease (Swanson et al. 2012). However, to date, there seems to be more knowledge about the bioactivity of DHA than EPA (Wells et al. 2016).
Dietary changes over the past decades have led to an increasing n-6 /n-3 PUFA ratio, which have been associated with chronic inflammatory diseases (Patterson et al 2012). The optimal n-6 /n -3 PUFA ratio is not known, but it is thought that our ancestors survived on a diet with a ratio of 1:1 to 4:1, whereas the average proportion among the western population today is more than 15:1 (Oliver et al. 2020).

2.1.2. Stress and enhanced lipid accumulation

The production of lipids in microalgae can be greatly affected by cultivation conditions during growth by inducing various stress conditions (Sun et al. 2018). When microalgae are exposed to extreme environmental conditions, such as nutrient limitations, temperature stress or light stress, lipids act as an energy-rich carbon storage for the cells to survive of. Nutrient related stresses such as nitrogen, phosphorous and sulfur limitation have shown to have positive effects on the accumulation of lipids in many species (Shi et al. 2020). For example, the lipid content of *Chlorella zofingiensis* increased significantly from 24.1% to 65.1% under nitrogen deficiency. There was also an increase in lipid content when cultivated in a phosphorus deficient medium, from 24.1% to 44.7% (Feng et al. 2012).

Temperature is a crucial factor for algal growth and their biochemical composition. Temperature stress has been found to both increase growth and lipid production in many species. However, it seems to especially affect the composition of the lipids, more than the total yield. Low temperatures have been reported to increase PUFA production while high temperatures have been correlated with higher carotenoid production (Shi et al. 2020). For instance, Safdar et al. (2017) reported an enhanced DHA content (34.9-36.4% of total fatty acids) in *C.cohnii* at temperatures of 15–20 °C, which was an increase of almost 40% than at 40 °C. The overall lipid content also decreased when the temperature increased. At 20°C the highest lipid content was observed (26.3% of dry weight) which was 60% higher than at 40°C.

Kwak et al. (2016) studied eight different microalgal strains and the changes in lipid content during various stresses (nitrogen starvation, high temperatures, pH shifts and high salt concentrations). It was reported that the combination of two stress conditions highly improved the lipid production (25% to 54%), compared to just one. However, three of more stresses at the same time reduced the lipid content of all strains.

The stress-response mechanism can be used as a tool to increase lipid production of many microalgal species. However, stressful conditions often have a negative impact on the growth which lowers the total product yield. This makes it important to find an optimal balance between growth rate and lipid content for an economically sustainable production (Shokravi et al. 2020).

2.1.3. Applications and market

As previously stated, DHA and EPA are mainly derived from marine sources (Peltomaa et al. 2018) and human consumption of seafood has increased. However, due to global warming and overfishing, ocean ecosystems are declining and fish stocks are becoming insufficient, and cannot provide a sustainable source of DHA and EPA (Remize et al. 2021). The development of a sustainable mass-scale production of EPA and DHA from microalgae could therefore address a global problem of the imbalance between fish oil supply and market demand.

To date, algal oils stand for roughly 2% of the human DHA/EPA consumption. However, the global market has been growing fast in recent years due to the increasing awareness about the health benefits omega-3 fatty acids can have on humans. Furthermore, food regulations are now favoring the use of DHA in infant formula, which has increased the demand. Oils from *C. cohnii* and *Schizochytrium* sp. are used as nutritional supplements or to enrich food and feed around the world. They can be used as aquaculture feed or as ingredients in animal feed to obtain eggs, chicken, and other animal products enriched in DHA.

Even though microalgal oils are mainly applicated into infant formula, the development of oils for adult consumption is in progress (Lopes da Silva et al. 2019).

2.2. Microalgae as a source of proteins

Globally speaking, plants are currently the major source of proteins for food and feed. The increasing food demand with the rising population could be helped by increasing cultivation area and optimize crop production. However, this may lead to further environmental problems such as land and forest degradation and loss of biodiversity. Moreover, animal-based proteins which are the main protein source of the Western diet, rely on suitable and cheap plant-based proteins for feed. (Caporgno & Mathys 2018).

Microalgal biodiversity provides a range of biological resources, including proteins and was proposed as a sustainable protein source already in the 1950s (Becker 2007). Proteins are important components of the structure and metabolism of the cells. They are essential elements of membranes as well as in the light-harvesting complex, including enzymes engaged in photosynthesis (Barkia et al. 2019). The protein content varies greatly among microalgal groups and the specific differences in protein content are linked to variabilities in their cell wall structure, photosynthetic apparatus and strategies for storage. For instance, the algae of the phylum *Cyanobacteria* and *Cryptophyta* have the highest protein contents, due to their protein rich cell walls (Lafarga 2020). An important factor determining the quality of proteins is their amino acid composition. When it comes to microalgal proteins, several species are excellent sources of essential amino acids (EAA). EAA are amino acids that cannot be synthesized by the human body and therefore must be supplied through the diet. EAA include isoleucine, leucine, lysine, cysteine, phenylalanine, tyrosine, valine, methionine, tryptophan, threonine and histidine (Amorim et al. 2020).

Commonly cultivated species such as *Arthrospira maxima, Arthrospira platensis* and *Chlorella vulgaris* are able to compete both quantitatively and qualitatively with protein sources such as soybean, egg, corn and cow's milk (table 2). According to recommendations from WHO/FAO these microalgae have well-balanced EAA contents necessary for human consumption.

Moreover, microalgae produce high protein yield, 4–15 tons/Ha/year compared to the yield of some terrestrial crops, 1.1 tons for wheat, 1–2 tons for pulse legumes and 0.6–1.2 tons for soybean (Koyande et al. 2019). When comparing microalgae-based proteins to some animal-based proteins, microalgae have significantly lower land requirements: <2.5 m^2 /kg protein, compared to 47–64 m2 for pork, 42–52 m^2 for chicken and 144–258 m^2 for beef (Caporgno & Mathys 2018).

Table 2. Content of EAA in different microalgae proteins and proteins from different sources, and required values from FAO/WHO for adults (g/100g of protein).

Source	Protein (%/100g)	Ile	Leu	Val	Lys	Phe + Tyr	Met + Cys	Trp	Thr	His
FAO/WHO		4.0	7.0	5.0	5.5	6.0	3.5	1.0	4.0	n.d.
Egg	12.4	6.6	8.8	7.2	5.3	10.0	5.5	1.7	5.0	2.4
Corn	10	3.4	11.2	4.9	3.2	8.9	3.3	4.4	3.7	n.d.
Soybean	37	5.3	7.7	5.3	6.4	8.7	3.2	1.4	4.0	2.6
Cow's milk (3% fat)	3.51	4.7	9.5	6.4	7.8	10.2	3.3	1.4	4.4	2.7
Arthrospira maxima	60-71	6.0	8.0	6.5	4.6	8.8	1.8	1.4	4.6	1.8
Arthrospira platensis	46-63	6.7	9.8	7.1	4.8	10.6	3.4	0.3	6.2	2.2
Chlorella vulgaris	51-58	3.8	8.8	5.5	8.4	8.4	3.6	2.1	4.8	2.0

No data: n.d.
Table references: (Gard et al. 2010; Harper 1981; Amorim et al. 2020; Chronakis & Madsen 2011; Swedish National Food Administration n.d.).

The protein digestibility is also an important factor in determining the quality of proteins. Even if the amino acid profile is excellent, the nutritional value of the protein can be low if the digestibility is low, because of poor bioavailability. Majority of the algal proteins are immobilized inside cells and the rigid cellulosic cell wall of many microalgae (e.g. green algae such as *Chlorella* sp.) constitutes a problem for digestion of the biomass, due to humans and other non-ruminants not being able accessing the encapsulated intracellular proteins and digest it. Therefore, extraction procedures to disrupt the cell walls are often necessary, by which the proteins as well as other components can be accessible to different digestive enzymes. Microalgae part of the phylum C*yanobacteria* such as *Arthrospira* sp. are unique because they do not have a thick cellulosic cell wall which makes them easy to digest and not in need of processing. Another example of this includes *Dunaliella* sp. which have no cell wall at all (Barkia et al 2019; Chronakis & Madsen 2011).

2.2.1. Stress and accumulation of proteins

The protein productivity of microalgae can be improved by certain cultivation strategies. Nevertheless, the protein content of microalgae is at its highest when cultivated under non-stress conditions, while environmental stresses can increase the accumulation of lipids and carbohydrates. The most crucial factor for obtaining a high protein content is sufficient amount of nitrogen (Amorim et al. 2020). However, even if nitrogen availability is crucial and strongly related to EAA synthesis in microalgae, increased levels do not automatically lead to higher EAA content. The major response to nitrogen deprivation in microalgae is to concentrate cellular nitrogen by which EAA synthesis can continue. Nevertheless, long term nitrogen limitation will lead to decrease of amino acids and proteins. For instance, short nitrogen starvation resulted in increased synthesis of all EAA in microalgae *Dunaliella salina*, while high levels lead to a decreased content (Sui et al. 2019).

2.2.2. Applications and market

It is apparent that the nutritional value of microalgal proteins is comparable with several conventional protein sources in terms of both protein quantity and quality, and have potential as an alternative protein source for human nourishment.

Protein-rich microalgae can be used in different food products as either a whole-cell ingredient in which it functions as inactive fillers, or added as protein extracts, which may have useful functions. Some microalgae proteins and hydrolysates have properties such as foaming, emulsifying, gelation, as well as water and fat absorbing functions (Grossman et al. 2020; Caporgno & Mathys 2018).

The global microalgal market is currently dominated by *Chlorella* and *Spirulina* spp. due to their high protein content and because they are easy to cultivate. They are commonly marketed as supplements in forms of tablets, capsules, powders and liquids, but are also incorporated in a number of food items (Chronakis & Madsen 2011). However, the utilization of proteins from microalgae into foods has been limited so far and this is mainly due to the presence of components such as chlorophyll which can affect color and taste of the food products (Barkia et al. 2019).

In recent years, the use of microalgal peptides for innovative functional foods has gained increased interest. Peptides are short chains of amino acids, up to 20-30 amino acids long and are inactive in their primary structure of proteins. They become active when they are digested in the gastrointestinal tract or when a food is processed (e.g., ripening, cooking or fermentation) (Caporgno & Mathys 2018). They are generally defined as bioactive peptides and can interact with many macromolecules and compounds, having anti-microbial, antioxidant, anti-tumoral and anti-inflammatory functions (Apone et al. 2019). The main challenge with production of peptides for food is the high production cost.

They are currently mainly generated from low-value food sources, including processed co-products from plants and animals. Microalgae have been identified as great sources of bioactive peptides but are yet to be commercialized (Lafarga 2020).

2.3. Microalgae as a source of carbohydrates

Through the use of the light harvesting reactions of the photosynthesis, microalgae take in and convert the light energy from the sun and use it further to convert water and CO^2 to carbohydrates, either formed in the cytosol of prokaryotes or inside the chloroplasts of eukaryotic microalgae. Carbohydrates include a broad range of simple sugars (monosaccharides) and their polymers, di-, oligo-, and polysaccharides (Markou et al. 2012). The function of carbohydrates in algae are mainly of two different categorizes, the first is the carbohydrates found as structural components in the cell walls and secondly as storage components that are found either in the cytosol or inside the chloroplasts (Geider & La Roche 2002).

Less mentioned function is that carbohydrates in the form of polysaccharides also are involved in the cell communication, and cellular recognition sites. Another type of polysaccharides are exopolysaccharides (EPS), they exist in the extracellular medium and without any covalent linkage connecting them to the cell wall, their roll for the cell is unclear, but it is hypothesized that they play a role in environmental adaptation, preventing desiccation or are factors to control photosynthetic activity, or they are involved in colony formation (Levasseur et al. 2020). Microalgae species differ in their carbohydrate content of the cell walls. For example, a rigid cell wall can be provided by high levels of polysaccharides, for example glucose and mannose in *C. zofingiensis.* The cell wall of *Tetraselmis suecia* contains the sugars galactose, arabinose, rhaminose, mannose and xylose. Cell walls made of peptidoglycan (e.g., *Arthrospira* sp.) are not as rigid and easier to degrade. The firmness of the cell wall affects the accessibility of the nutrients to different digestive enzymes (Corrêa et al, 2021). The type of storage carbohydrates are species specific; cyanobacteria synthesize glycogen, red algae synthesize a starch and glycogen hybrid known as floridean starch and green algae make starch (Markou et al. 2012).

The carbohydrate content of microalgae differs also with cultivation conditions, e.g., nutrient accessibility, light intensity, and CO_2 concentrations, which can influence the algae to form either carbohydrates or lipids (Cheng et al. 2017). The amount of carbohydrates can similarly to lipids, differ significant and under normal conditions both of those groups of molecules are often found to be in the range of 10-50% of the cell weight in dry condition.

But microalgae can under certain condition if it is a suitable species accumulate and reach very high values (Geider & La Roche 2002).

When microalgae are limited in nitrogen, most of the carbon is used to produce lipids or carbohydrates, instead of proteins. Phosphorous, sulfur, carbon and trace mineral limitations also influence the accumulation of carbohydrates. Temperature is another factor that also has been reported to affect the carbohydrate level in microalgae. For instance, in *Spirulina* sp., increased the carbohydrate level by 50% when the temperature was risen from 25 to 40°C (Cheng & He 2014).

One of the molecules that function as carbon storage is glycogen, especially in prokaryotic cyanobacteria and mobilization of this molecule is done with the enzyme glycogen phosphorylase. The amount of the enzyme and its activity are increased in dark environments and decreased in the presence of light. Limited exposure to light of cyanobacteria results in increased levels of accumulation of pigment and a high exposure and intensity of light increase formation of polysaccharides. There seem to also exist differences in how both the eukaryotic microalgae and cyanobacteria alter their content of both polysaccharides and pigments, where changes in photoperiod and photosynthetic efficiency impact how the organisms accumulate these two groups of molecules and the rate in which they are produced. Species such as *Asterionella formosa* and *Scenedesmus protuberans*, both eukaryotes, adopt and adjust their content of pigments only after changes of irradiance levels and not to the length of the day. The prokaryotic cyanobacteria on the other hand can optimize their storage of polysaccharides, so that they have a higher rate of forming saccharides and can therefor use this stored reserve for growth in the dark (Kromkamp 1987).

2.3.1. Sugar content and application

The microalgal carbohydrates consists mainly of glucose, starch, cellulose, and different polysaccharides (Chew et al. 2017), and most microalgae lack lignin and hemicellulose which makes them well suited to be used as biomass for fuel production. Some even lack cellulose or have no cell wall at all. So, extraction often become less of a work burden, due to no pretreatment needed for lignin removal or conversion (Markou et al. 2012). Starch and glucose are mainly used in the industry, where they are fermented to biofuel production (Chew et al. 2017). Carbohydrates is the least energy abundant group of the three-organic groups, protein, lipids, and carbohydrates, containing 15,7 KJg-1, compared to 16,7 KJg-1 respectively 37,6 KJg-1 for protein and lipids. Even so it is still one of the preferred compounds in the processing of biofuel, where it is used for producing fuel as, bioethanol, biobutanol and biohydrogen (Markou et al. 2012; Geider & La Roche 2002). Polysaccharides from microalgae are more suitable as ingredients in foods with therapeutic effects (Chew et al. 2017). The carbohydrate composition of some microalgae species can be seen in table 3, where the most abundant are foremost of the carbohydrates are glucose, galactose, mannose, rhamnose and xylose. Different microalgae have great variety in which of the carbohydrate that is most abundant.

For instance, *Chlamydomonas reinhardtii* has the highest amount of glucose but no content of xylose, while this is found in the rest of species listed. *A. platensis* has half of its content of carbohydrate as glucose, no content of galactose and the highest listed content of rhamnose (22.3%), of the listed species (Markou et al. 2012).

Table 3. Composition of the most abundant carbohydrates of some microalgae species.

Sugar	*Chlamydomonas reinhardtii*	*Chloroccum sp.*	*Dunaliella tertiolecta*	*Nitzchia ciosterium*	*Phaeodactylum tnicornutum*	*Arthrospira platensis*
Glucose (%)	74,9	47	85,3	32,6	21,0	54,4
Galactose (%)	4,5	9	1,1	18,4	8,9	-
Mannose (%)	2,3	15	4,5	16,8	45,9	9,3
Rhamnose (%)	1,5	-	5,5	7,7	8,6	22,3
Xylose (%)	-	27	1,0	7,0	7,5	7,0

Table reference: (Markou et al. 2012).

2.4. Microalgae as a source of pigments

Microalgae have been existing for over 3,5 billion years and adapted to a great number of environments. Today we can find them in every aquatic environment, across the entire planet and there exist over hundreds of thousands of different species, some adapted to the cold climate of the snowy arctic, others in acidic water found inside mines or in the deserts (Novoveská et al. 2019). In their natural habitat, they are exposed to numerous environmental factors and conditions, exposing them to harm, such as high oxygen and radical stress. This made them develop several systems for protection from factors such as free radicals and reactive oxygen species (Faraloni & Torzillo 2017; Novoveská et al. 2019).

The main function of pigments such as carotenoids is to absorb light, within the wavelength of 400-550 nm, in order to harvest the energy for the reactions of the photosynthesis and to protect the organism from excessive damage done by high amounts of light (ibid.). Carotenoids are pigments with colors of yellow, orange, red and deep red, which is depending on the number of conjugated double bonds. The molecules are made up of and derived from tetraterpenes, formed of 8 isoprene units bound together, forming a polyene structure consists of a chain of 40-carbons. Terpenes are the largest family group of natural organic metabolites and products, consist of over 1000 carotenoids occurring naturally in nature. They are found in over 600 different organisms like algae, fungi, bacteria, archaea, and plants.

Humans cannot synthesize carotenoids and they are therefore needed from other sources to obtain sufficient daily amount for our health. Carotenoids can be divided into two groups of molecules, the oxygen-containing xanthophyll's, such as zeaxanthin and astaxanthin, and the carotenes being pure hydrocarbons and oxygen-free, such as β-carotene, and lycopene (Novoveská et al. 2019). Within the nomenclature, carotenoids are classified by their composition of the end groups of both sides. There are seven different end groups and each group has got a denoted Greek letter (β, γ, ε, χ, κ, φ, ψ), and by having two ends they are designated two letters in their name. For instance, α-carotene has the formal name β, ε carotene, due to the presence of one β ring and one ε ring in this molecule. β-carotene with its formal name β, β carotene, has two β rings as end groups. The names can be a bit more complicated, as seen with the molecule astaxanthin which has the formal name 3,3'-dihydroxy-β, β-carotene-4,4'-dione, so it is easier to just say astaxanthin. As for microalgae they are having the capability to produce carotenoids from all known xanthophylls of terrestrial plants and they can even produce additional pigments that are only found in microalgae and some yeasts, such as astaxanthin, diatoxanthin, diadinoxanthin and fucoxanthin. The type of pigments found in algae vary greatly with species and this makes pigments work as chemotaxonomic markers for categorization of microalgae into classes (Chekanov et al. 2019; Novoveská et al. 2019).

2.4.1. Function of primary and secondary carotenoids

Carotenoids are in microalgae grouped by primary carotenoids and secondary carotenoids. The primary carotenoids such as β-carotene and xanthophylls are normally found in the chloroplast and secondary carotenoids can be found dissipated in the cell, either in lipid vesicles in the stroma of a plastid or in the cytosol. Some carotenoids are synthesized in the chloroplast and can then be exported into the cytoplasm, which makes them findable anywhere in the cell.
Primary carotenoids are directly linked to the light-driven electron-transport and this is always strictly controlled by the cell. The secondary carotenoids are on the other hand not directly linked to the photosynthesis (Chekanov et al. 2019; Novoveská et al. 2019). Instead, secondary carotenoids are accumulated as protection and counteract damage from environmental stress, such as excessive light and oxidative damage, and extreme temperatures. This makes microalgae able to survive under extreme harsh conditions (Chekanov et al. 2019; Faraloni & Torzillo 2017). Not all microalgae are producers of secondary carotenoids. The phenomena of producing secondary carotenoid types are called secondary carotenogenisis (Chekanov et al. 2019).

2.4.2. Stress and accumulation

There are many factors of stress that can cause microalgae to accumulate carotenoids, factors such as high light, temperature changes and nutrient deficiency (table 4) (Faraloni & Torzillo 2017; Chekanov et al. 2019). One stress factor that is seen in all the listed microalgae is that high intensity to light cause stress and improve accumulation of various carotenoids.

Table 4. Carotenoid accumulation and stress factors of some microalgal species.

Microalgae	Carotenoids	Stress factors
Chlamydomonas reinhardtii	Zeaxanthin; lutein	High light, sulphur starvation, anaerobiosis
Dunaliella salina	β-Carotene	High light, high temperature
Haematococcus pluvialis	Astaxanthin; canthaxanthin; lutein	High light, nitrogen starvation
Isochrysis sp.	Diatoxanthin; fucoxanthin	High light, nutrient starvation
Phaeodactylum tricornutum	Diatoxanthin; fucoxanthin	High light, nutrient starvation
Scenedesmus sp.	Lutein; β-carotene	High light, nutrient starvation

Table references: (Faraloni & Torzillo 2017)

2.4.3. Applications and market

Microalgae pigments and natural pigments tend to often be less stable than synthetic ones, but often compose higher nutrient values and are also more environment friendly due to the production of the synthetic pigments often comes with high industrial use of toxic chemical solvents, that are often by-products of petrochemicals, and they also often leak out and do not get disposed of correctly (Nemer et al. 2021). Several researchers have focused on the application of microalgal carotenoids, and it has been confirmed that carotenoids can have important roles in the prevention or treatment of cancer, cardiovascular diseases, arthritis, neurological disorders, and other diseases. This is due to their antioxidant, anti-inflammatory and antitumoral affects. The demand for microalgal pigments is high, especially β-carotene which easily reach a price of 700 €/kg, whereas synthetic β-carotene cannot attain more than half that price (Guedes et al. 2011). Natural β-carotene is preferred to be used because it contains a mix of cis and trans β-carotene, and especially the cis configuration has been found to possess anticancer properties (ibid.).

The global market for carotenoids was valued to a sum of $1.24 billion in 2016 and are expected to increase to $1,53 billion by year 2021 (Novoveská et al. 2019).

There are many industries that have practical uses for carotenoids and applications can be found in food, feed, nutraceutical, medicine, and cosmetics. Carotenoids are said to be safe to use and with their nutritional value, increased in popularity over the synthetic ones. For instance, in the feed industry, pigments are used in the poultry industry and aquaculture, due to their colouring properties. For instance, by giving egg yolk a more desired brighter yellowish colour and giving fish and crustaceans, such as salmon, its expected pink/red shade. (Novoveská et al. 2019). The aquaculture is also one of the fastest growing food industries today, and already does it stand for almost half the fish consumed in the world and it is expected to grow future in popularity (Ottinger et al. 2016).

2.5. Microalgal foods

As the global population increases and the world confronts climate changes, the demand for sustainable food and feed sources increases. Microalgae have been showing potential as sustainable sources to balance the demands (Khan et al. 2018). Microalgae as food is not a new idea. Edible microalgae such as *Nostoc*, *Arthrospira* and *Aphanizomenon* spp. have been used by indigenous populations as food for thousands of years. However, the cultivation of microalgae is only a few decades old (Spolaore et al. 2006).

The most commonly cultivated microalgae for human nutrition are *Chlorella, Arthrospira, Dunaliella, Nannochloris, Nitzschia, Crypthecodinium, Schizochytrium, Tetraselmins,* and *Skeletonema* spp. Most microalgae food items are (i) composed of dried microalgae, (ii) supplemented with microalgae or (iii) supplemented with a certain compound extracted from microalgae, such as PUFAs or pigments (Lafarga 2020). Dried microalgae are primarily sold as dietary supplements in the forms of powders, tablets, capsules, or liquids and are advertised as “superfoods” (Lafarga 2019).

The regulation of microalgae-based food products and food supplements vary among countries. In the EU, novel foods and food ingredients are regulated by the Novel Foods Regulation No. 258/97 (Sidari & Tofalo 2019). A novel food has been defined as a food that has not been widely consumed in the EU before 15 May 1997 (European Commission n.d.). *A. platensis, C. luteoviridis, C. pyrenoidosa* and *C. vulgaris* were sold and consumed before this date and are therefore not regulated by the 258/97 regulation (Hayes et al. 2018). Examples of microalgae being approved under the regulation is DHA and EPA-rich oils from microalgae *Schizochytrium* sp. which have been approved to be placed on the EU market since 2003 as a novel food ingredient (Turck et al. 2020). Moreover, β-carotene from *Dunaliella* sp. and DHA from *C. cohnii* are approved as novel food ingredients and *Tetraselmis chuii* as a novel food (Hayes et al. 2018, Sidari & Tofalo 2019).

The number of microalgae incorporated foods available on the market has significantly increased during the past years (Lafarga 2019). Dried biomass or extracts of *Chlorella* sp. have seen to be used as additives in the traditional Japanese dish natto (fermented soybeans), liquors, vinegar, green tea, candies, etc. (Chronakis & Madsen 2011). Other examples of microalgae enriched products currently available include baked goods such as bread and cookies, pasta, beverages, breakfast cereal and extruded snacks (Koyande et al 2019) and as previously stated, microalgae derived DHA has become highly popular as a supplement in infant formula (Lopes da silva et al. 2019). Microalgae have not only gained attention as a food source for human consumption, but are also used as feed in aquaculture and to feed ruminants, pigs, poultry and other livestock (Koyande et al. 2019).

Microalgae incorporated foods still encounter some challenges, often in terms of barriers related to regulatory authorities, as well low production capacities and high costs of production. Moreover, the intense and often green colour along with the fishy taste and smell of microalgae are perceived as undesirable among many consumers and limit the applications (Caporgno & Mathys 2018). Several experiments to modify or incorporate algae into known food items have been made, by using various processing methods such as heating, baking, mixing (Becker 2007). However, mainly small amounts of microalgae and derived compounds have been successfully incorporated into foods so far and these small amounts did not increase the macromolecular composition in any notably way (Caporgno & Mathys 2018). For instance, with higher additions of microalgae into bread, the dough taste and consistency became unappetizing (Becker 2007).

In the Asian part of the world, microalgae in foods are common since many traditional dishes include algae. However, in Western cultures it is not yet as accepted and common and several strategies for trying to mask the colour as well as the taste have been made (Lafarga 2019).

2.6. Cultivation of microalgae

Microalgae cultivation possesses advantage of not requiring any fertile land or high-quality water. They do not compete for water that are intended for human usage as they do not require freshwater to be cultivated (Santos-Sánchez et al. 2016). Moreover, microalgae cultivation does not require any herbicides or pesticides (Tan et al. 2020) and cultivation can take place on land where other agricultural activities can't take place, such as seashore lands, deserts or in semi-desert regions (Garcia-Vaquero 2021).

Microalgae can be cultivated either phototrophically (with light and CO_2), heterotrophically (with organic carbon and absence of light) or mixotrophically (with light, CO_2 and organic carbon) of which the first is the most commonly used condition (Rios Pinto et al. 2021). As aquatic organisms, microalgae require water along with a number of nutrients for growth (Pires 2015). 65-85% of the dry weight of microalgae are made up of carbon, oxygen and hydrogen, which are provided through photosynthesis under photoautotrophic conditions (Bauer et al. 2021). For heterotrophic microalgae that grow in the dark, an organic form of carbon (i.e., acetate or glucose) is needed. Mixotrophic microalgae can use both organic and inorganic carbon sources (Jareonsin & Pumas 2021).

Biomass composition of microalgae varies with species, but the average composition besides carbon, hydrogen and oxygen are generally 7.6% nitrogen, 2.5% sodium, 2.3% potassium, 1.4% sulfur and 1.1% phosphorous. These values can be used as a guideline for the nutrient composition of the medium (Bauer et al. 2021). For mass production of microalgae, different reactor types are available and they are commonly divided into open and closed systems (Panahi et al. 2019).

2.6.1. Open System

The open systems are simple designs in the form of lakes, tanks and various types of ponds such as shallow raceway types, circular ponds and inclined ponds (Panahi et al. 2019). One of the first efforts to cultivate and mass-produce microalgae was made by using open raceway ponds. The open systems are still the most widely used approach for cultivation of microalgae and has been preferred due to low capital and operational costs, and low energy demand for culture mixing. However, a disadvantage with the system is the lack of control over growth factors such as culture temperature and evaporation losses which lead to lower biomass productivity. Moreover, open systems are also susceptible towards adverse weather conditions and it is difficult to obtain contamination control, which is of importance for food applications (Narala et al. 2016). The difficulties in species control restricts it to cultivation of microalgae that are adapted to live in extreme environments, e.g., high alkalinity (*Arthrospira* sp.), high concentrations of nutrients (*Chlorella* sp.) and high salinity waters (*Dunaliella* sp.) (Vieira Costa et al. 2019).

The open systems are suitable for producing biomass or cell components when the costs of construction and production constitutes major restrictions. Another factor to consider is the climate. Most microalgae species can be cultivated at 15-30°C, with an optimum of 20-25°C (Ras et al. 2013) and a large part of microalgae for human nutrition are cultivated in very large open ponds (5.000 - 5.000.000 m2) in tropical and subtropical zones in Asia, followed by Australia and Israel. However, cultivation in cold temperature climate zones has been investigated scarcely and it has been suggested that closed systems known as photobioreactors (PBRs) are a

more suitable for colder climates (Schade & Meier 2020; Chronakis & Madsen 2011).

2.6.2. Closed system or photobioreactors (PBRs)

PBRs have been developed as an alternative to open systems in order to overcome issues such as environmental disturbances. There are several types of PBR designs, with mainly tubular, plastic bag, flat plate or column type containers (Grossman et al. 2020), provided with controlled gas exchange, internal or external light sources and mixing of the media (Singh et al. 2015).
This system require relatively little space, can enhance the light availability and are less susceptible towards contamination issues compared to open systems (Narala et al. 2016). Moreover, PBRs are more efficient with greater biomass obtained than open systems, i.e., 1.25 kg/m3/day compared to 25 g/m3/day (Bellou et al. 2014), due to increased control over factors such as temperature, evaporation losses and CO2 shortage (Singh & Das 2013).
However, even if PBRs are far more reliable than open systems, they are significantly more expensive and more difficult to scale up (Lafarga 2020).
PBRs are used for production of pigments such as astaxanthin and beta-carotene from *D. salina* and *Haematococcus pluvialis*. They are also used for production of omega-3 PUFA containing lipids. This is possible since the high prices of these products can cover the production costs (Bellou et al. 2014).

2.7. Extraction of compounds from microalgae

Compounds of microalgae have many different uses in the industry, in food, textile and medicine among others, and there are a wide range of species that have high content of those valued compounds. But the methods to extract them differ with variety of yield, cost, and time. Some of these methods are reviewed here. The different methods for extraction are often, when described, divided into two groups: mechanical and physical techniques, and non-mechanical. The foremost purpose of most of the methods is to disrupt the cell wall and membranes to get the intracellular content to leak out of the cell. However, there are also methods where the goal is to keep the cell intact so that the organism can continue producing further products, so call milking of the cell. Many of the methods are also used in combination, for instance by using solvents or enzymes before or after further methods (Günerken el al. 2015; Hejazi & Wijffels 2004).

The extraction of lipids from microalgal cells is often done by mechanical disruption of the cell walls, following by use of organic solvents or solvent mixtures, such as chloroform-methanol and hexane-isopropanol. To further separate PUFAs, additional processing steps, including fractional distillation or de-

waxing is made. However, these fractions can contain impurities, have bad taste and smell, and to increase the quality and shelf life and make them suitable for consumption, further purification of the fractions is needed. This includes filtration, bleaching, deodorization, polishing, and antioxidant addition. The process of extracting carotenoids is also often performed by using solvents or more environmentally friendly methods, such as microwave irradiation, ultrasonication or enzyme-treatment extraction (Barkia et al. 2019). Microalgae proteins have poor digestibility in their raw form which is why it has been important to develop improved methods for protein extraction. The proteins are mainly extracted by using mechanical methods such as bead milling or ultrasonication to break the structure of the cell wall in combination with chemical treatment (aqueous, acidic, and alkaline treatments) and centrifugation (Bleakley & Hayes 2017). Polysaccharide extraction has typically been performed using hot water. This method is simple and easy to perform in large scale, however, it is time-consuming and has low efficiency. Therefore, other extraction techniques such as microwave irradiation, ultrasonication, and enzyme-treatment extractions have gained increasing popularity to enhance the yields (Barkia et al. 2019). Many of the methods of mechanical extractions have shown to have great potential in the field of extraction of bioproducts from microalgae and especially extraction of biosynthetic pigments. Several of these methods have also shown evidence of great yield and the possibility of separation of different compounds such as protein, polysaccharides, lipids, etc. (Nemer et al. 2021)

2.8. Mechanical / physical extraction methods

2.8.1. Bead milling

Bead milling (BM) is a process of which the biomass is going through a milling machine, with the purpose to break the structure of the cell wall. The machine is often operating together with the use of beads in the grinding chamber. The beads can be of different material and size, where the density of the beads and size can have a great difference for the outcome of disruption of the cell wall of microalgae. The purpose to fracture or break the cell wall, is to access and extract the intracellular content of the algae. The destruction of the cell walls by applying mechanical force through BM is highly efficient. BM is effective against medium considerable and rigid cell walls and can be used in large scale industries and the machines can be loaded with a high amount of biomass.

The process is having a low labor requirement and is carried out in a single-pass operation. One backside of the process in the machinery is that the biomass is exposed to problems with friction, which can cause high temperature, but some machines can often avoid this with the use of integrated water cooling. By using

active water cooling, one can avoid and prevent rise of excessive heat that can cause denaturation, and other unwanted effects on the extracted compounds. Some drawbacks with mechanical methods including BM are that there are many parameters that can impact the yield and the quality of the product, such as if the biomass is processed in a wet or dry state, as well as the morphology of the species or strain. Other parameters are the optimization of the BM and the parts of the machinery, such as the blades, bead, rotors, stelar, as well as the speed and the number of rotations per minute. All the different parameters of BM can be time consuming to tune and give unpredictable results. Energy consumption can be high and fine-tuning is often required to ensure efficacy and efficiency of the process. Fine-tuning is often selected for the specific microalgae that is used and what works with one alga can be different for the next one. (Nemer et al. 2021; Günerken el al. 2015)

2.8.2. High speed homogenizer

High speed homogenization (HSH), is a device that stirs and rotates its stator-rotor or blades at a high speed, measured in rounds per minute (rpm), this causes rupture and disruption through that the mechanical application of shear stress and the action also causes cavitation, gap of vacuum which causes burst of energy. HSH, is widely used in the industry, because it only needs a few seconds of action. It can be used on dry matter or in liquid solutions and sometimes it is also used together with solvents, either used as pre- or post-treatment to obtain a higher yield. But the extended use of solvent is a question of whether the action will have effect on the type of microalgae used or it is just becoming an extensive cost (Günerken el al. 2015).

2.8.3. High-pressure homogenization

By the use of high-pressure homogenization (HPH), a liquid suspension containing microalgae is treated with pressure. The suspension is forced under high pressure through a valve and an orifice, where the start of the valve has a wide passage and the liquid is forced through a narrow opening. With a sudden pressure drop after the passage, this action is causing collisions, turbulence and the formation of “high-speed suspension jets”, which disrupts the cells (Nemer et al. 2021; Diels & Michiels 2006). The system of HPH is highly scalable, smaller than that of the BM system. They can process cells with highly rigid cell walls without any major problems.

Drawbacks of HPH, is that the action of the machine can cause or create problems with fine particles and debris that can plug the down streaming of the systems packing filters. This can thereby halt the processes and bring up the cost of extraction. The process is fast, but another problem that can occur is problems from friction induced heat, but this can be treated and taken care of after (post-extraction

cooling) the extraction process. Moreover, the apparatus can be both expensive to purchase, maintain and repair (Nemer et al. 2021).

2.9. Electromagnetic and thermal extraction

2.9.1. Microwave irradiation

By using microwaves to extract products from microalgae, the irradiation causes the cell walls of the algae to disrupt by the electromagnetic effect caused by the microwave irradiance, promoting heating. The heat promotes the expansion of the intracellular liquid and this itself causes the cells to disrupt or burst, breaking the cell membrane and wall, and releasing the intracellular content. Extraction using microwave irradiation is both described as simple and an easy method to scale up and to operate. The optimal value to use and operate is of 2450MHz. The method can also be used together with either solvents or enzymes (MAE microwave-assisted extraction), this to implement better outcome and greater yield (Corrêa et al. 2021).

The use of microwave irradiation is not appropriate for extraction of volatile compounds, but has been reported as effective for lipid extraction. Moreover, MAE has been reported to have lower extraction time and operational costs than conventional extraction methods, being environmentally friendly, as well as having higher lipid extraction than other non-conventional methods such as ultrasonication (Corrêa et al. 2021; Zghaibi et al. 2019). Microwave irradiation was the most effective method for cell disruption of *C. vulgaris*, *Botryococcus* sp. and *Scenedesmus* sp., when compared to other extraction methods; BM, sonication, autoclaving and osmotic shock, followed by chemical extraction of the lipids (Lee et al. 2010).

2.9.2. Ultrasonication

Ultrasonication (USN), is the use of sound to disrupt cells. The sound and its vibrations are generated by a resonance rod or a sonar and the sound is resonated and transmitted through a material or a suspension of liquid together with cells of microalgae. The sound that is formed is of subharmonic frequencies several multipliers under the normal tone and it creates bubbles in the suspension which forms an alternating pressure field. The formed bubbles grow until a cavity are formed in the bubble and it collapses, producing energy that ruptures the cells of nearby microalgae in the liquid. The formation of the bubbles also increases pressure and temperature, close to the sonar (Greenly & Tester 2015; Nemer et al. 2021). USN system can have active cooling, which can be crucial when the favored component of extraction is thermolabile as e.g., pigments. Having ability to

minimize and prevent degradation and loss of function of the molecules because of thermal impact, will also have a great impact on the yield and cost.
The systems of USN are easier to control then the HPH system, are more precise, less labor intensive and the control of the systems such as the speed and direct impact on the cells, are more finetuned and therefore can prevent degradation of the extracted molecules. The system has the attributes of being of high reproducibility, giving the same product outcome repeatedly, and it is easy to calculate factors such as the percentage of cell disruption, time, energy, and costs of the manufacturing. The operation of the USN technique is also fairly easy to maneuver. Factors that make this technique less attractive is the area of effect, due to that the substrate must be at a close vicinity to the sonar or resonance rod, that makes the disruption happen. Moreover, the rigid cell walls of some species of microalgae can be harder to disrupt (Nemer et al. 2021).

2.10. Chemical / biological extraction methods

There are many chemicals that can be used in some way to interact with cells and their cell wall and membranes, for disrupting, weakening, deformation or inhibiting the structural components in microalgae. It can be chemicals such as solvents, acids or alkalis, detergents, antibiotics, enzymes, and many more. The method of choice varies depending on, if the purpose is for analyzing and quantifying the content in a lab, or searching for components to be used in large scale industries. Moreover, if one wants the method to be safer and lower the usage of toxic chemicals, implementation of greener methods and chemicals can be made (Corrêa et al. 2021).

2.10.1. Surfactants

Surfactants can both help with harvesting of the biomass and assist in cell disruption. Surfactants are amphiphilic organic compounds that have both a hydrophobic and a hydrophilic end. They attach themself to and interact with the surface of the cells. This interaction with the phospholipids of the cell membrane causes disruption through distortion (Corrêa et al. 2021; Huang & Kim 2013).
Surfactants are often long alkyl chain molecules with 8-22 carbons, classified into either ionic or non-ionic surfactant, and further subclassified as either cationic or anionic, and this can further depend on the pH of the solution its mixed into (Nakama 2017).

Surfactants have been reported useful for algal lipid extraction. There are several natural surfactants in microalgal suspensions, such as phospholipids, lipopeptides and monoacylglycerols. Various methods have been developed for the extraction of lipids from microalgae, however, only a few studies have researched the use of

surfactants or algal-based surfactants, to replace the use of organic solvents. More research is required to prove surfactants or algal-based surfactants useful, which could lower the use of organic solvents for extraction of microalgal lipids (Wu et al. 2017).

2.10.2. Solvents

Solvents are a structural diverse group of different chemicals used for dissolve, disperse and dilute other molecules and compounds, and the efficiency is dependent of the solvent and the solutions structure itself. Solvents can be classed as both organic and inorganic, depending on their composition and origin.
They can have different chemical polarity, polar and nonpolar. Polar solvents include chemicals as water, alcohols, acetic acid, etc., and nonpolar solvents that also cannot be mixed with water, consist of chemicals as hexane, benzene, and toluene, etc. (Bonventre 2014). Extraction methods using solvents are the most commonly used methods because they are simple and easy to scale up in the industrial field. Most often solvents like methanol and chloroform are used. The use of toxic and flammable solvents is a very important problem as they negatively affect health and environment (Santoro et al. 2019). Many of the solvents are categorized as volatile organic compounds (VOCs), and many of the commonly used solvents in the industry are petroleum chemicals. This is one of many reasons the industry and health departments are looking for replacing them with so called green solutions (Bonventre 2014).

2.10.3. Osmotic shock

The method of osmotic shock is when a solution of high concentration of either salt, dextran, or polyethylene glycol (PEG) is used to create a decrease of osmotic pressure which causes damage or the release of intracellular components from the cell (Corrêa et al. 2021). The suspension is then proceeded and the products and compounds are separated by centrifuging, causing the separation of the biomass from the liquid, this is often done together with solvents for further separation of the different masses (González et al. 2019).
Extraction of oil from various microalgal biomasses such as *C. reinhardtii, Botryococcus* sp., *C. vulgaris* and *Scenedesmus* sp., was achieved using the osmotic shock method, and concluded to be an easy and efficient extraction method of lipids from microalgae (Lee et al. 2010; Yoo et al. 2012). Further research is needed on extraction using the osmotic shock method from different microalgae species. Moreover, the possibility of using this technique at pilot- and production-scale has to be further investigated (Ranjith et al. 2015).

2.10.4. Enzymes

Extraction with enzymes is a highly selective method, due to the enzymes binding only to specific molecules. It is a very mild method to use, and it requires low energy (Corrêa et al. 2021). Enzymic lysis, disruption of cell membrane is widely used in different industries. Commonly used enzymes are cellulases, proteases, lysozyme, lipase, glucanases and phospholipase A1 (Alavijeh et al. 2020; Corrêa et al. 2021). The selection of a suitable enzyme is linked to the characteristics of the cell wall of the algae, which depend on species and growth conditions as well as harvesting and dewatering actions (Zuorro et al. 2016).

The enzymes affect and degrade cellulosic material, peptide- and ester bonds, and bonds of carboxy- and phosphor-diesters. Often are enzymes used together with other methods of extraction, such as bead milling, homogenization, and microwave irradiation, or as a pre-treatment before solvents are used. After the extraction, it is in generally required to apply separation methods such as ultracentrifugation, membrane separation or electrophoresis to purify the extracted compounds (Alavijeh et al. 2020; Corrêa et al. 2021).

Enzymatic extraction techniques are environmental-friendly, however, the drawbacks of the method compared to other chemical or mechanical techniques include low production capacity and long process time. Enzymes are also expensive, which limits their use in microalgae biorefineries. Moreover, the recovery of enzymes is an important issue (Corrêa et al. 2021; Shahid et al. 2020).

3. Conclusion

Microalgae as a source of high value compounds, have many uses in the industry, including as a food source. The world population is expected to increase by 2 billion in the next 30 years to reach 9.7 billion in year 2050 (UN 2019), meaning that the amount of food produced must greatly increase to cover the needs. Microalgae as food and nutraceuticals could be a larger part of our diet, counter the future increasing demand, as well as combat the rate of malnourishment in developing nations with already inadequate nutrient supply. Proteins, lipids and other essential nutrients from microalgae could establish an algae-based food industry to produce healthy foods. It has been observed that microalgae species have complete EAA profiles, are rich in essential fatty acids, therapeutic polysaccharides and other various bioactive compounds with known health benefits such as anti-inflammatory and antioxidative effects. As sources of nutritional and therapeutic compounds, they are attractive for the development of novel foods as well as to enhance nutritional values of conventional foods.

Application of microalgae-derived products have been growing exponentially in recent years. However, in the Western culture, microalgae foods still encounter some challenges. More research on how to successfully incorporate algal biomass into foods are needed, as well as technological developments to create an economically sustainable mass production, which currently is challenged by production costs, including cultivation systems and extraction processes. To date, PBRs are only suitable for the production of high-priced products, while open ponds are troubled by low productivity and contamination issues.

The expense of extraction processes has also been established as a major problem. Moreover, the use of traditional methods often using solvents, have negative effects on health, safety as well as the environment. Therefore, more greener technologies, are needed for the isolation of high-value microalgal products. Regardless of the challenges of mass-scale cultivation, there is an interest and demand for microalgae in foods, pharmaceuticals and in many other fields. To date, only a limited number of species are used for the production of a limited number of products. If the use of algal products as food should be able to become more widespread, it will require more research and development. Moreover, given that just a few species of microalgae out of hundreds of thousands, have been investigated and are used, there are opportunities of finding novel bioactive compounds.

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