High-Value Biochemical Products & Applications of Freshwater Eukaryotic Microalgae

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Abstract:
A shift in public perception of the health and nutritional benefits of organic supplements and skin care products has led to a surge in high-value products being extracted from microalgae. Traditional forms of microalgae products were proteins, lipids, and carbohydrates. However, in recent times the extraction of carotenoids (pigments), polyunsaturated acids (PUFA’s), vitamins, phytosterols and polyphenols has increased significantly. Despite the diversity of products most research has failed to scale up production to industrial scale due to economic constraints and productivity capacities. It is clear that the main market drivers are the pharmaceutical and nutraceutical industries. This paper reviews the high-value products produced from freshwater eukaryotic microalgae. In addition, the paper also considers the biochemical properties of eukaryotic microalgae to provide a comparative analysis of different strains based on their high-value product content.

Keywords: Microalgae, Eukaryotic, high-value compounds, biochemical compounds, nutraceuticals, pharmaceuticals.
Graphical abstract:
1 - Introduction:

The term *algae* represents a large group of plant-like organisms with sub-division classifications. The three major classifications are microalgae, macroalgae and cyanobacteria. These organisms can exist as either unicellular, multicellular, filamentous, or colonial structures. Microalgae can convert solar energy into chemical energy by fixing atmospheric CO₂, with an efficiency ten times than that of terrestrial plants such as oilseed crops (Barkia et al., 2019; Lauritano et al., 2016; Ranjith Kumar et al., 2015).

Cyanobacteria are gram-negative prokaryotic cells ranging from 1μm to 30μm and are classified into three basic morphological forms. Yousuf listed the three forms are: unicellular, filamentous without heterocysts and filamentous with heterocysts (2019). A heterocysts is described as a specialised cell that fixes nitrogen, which is believed to promote the survival of the cells under low nitrogen conditions (K. Y. Lim and M. Schenk, 2017). It is worth noting that the cyanobacteria’s ability to fix carbon and nitrogen impacts their potential for producing biofuels and chemicals is also desirable (Behrenfeld et al., 2001).

Macroalgae, also referred to as seaweeds, are a classification of multicellular living organisms that can exist independently or as part of a community within an ecosystem (Al-Dulaimi et al., 2021). These organisms are typically found around coastal regions and have traditionally been consumed in Asia. However, within the last decade seaweed consumption has rapidly increased around the world due to its health benefits (Amlund et al., 2019). Macroalgae are renowned for their ability to regulate their aquatic environment through the uptake of nutrients, providing protection to other aquatic life and offer structural support to the seabed, in a similar way to grass on dry land. One such example of macroalgae is the brown seaweed; *Laminaria hyperborea* that populates the coastlines of Scotland (Al-Dulaimi et al., 2021).
Microalgae or microphytes are defined as microscopic eukaryotic, unicellular, or colonial organisms. These organisms are usually found in aquatic environments; both freshwater and marine systems (Yousuf, 2019). They can be found in other environments such as frozen landscapes, deserts, or highly acidic surroundings (K. Y. Lim and M. Schenk, 2017). These organisms form the fundamental components of the world's food chain systems and are essential in regulating the global carbon cycle (Behrenfeld et al., 2001).

The extraction of high-value products such as carotenoids (pigments), Polyunsaturated Fatty Acids (PUFAs), lipids, sterols, vitamins, and antioxidants has been at the forefront of microalgae research over the past few decades. Dębowski et al., discusses new technologies for cultivation and extraction of multiple products, considering the advantages and limitations of each (2020). (Onumaegbu et al., 2019) the cultivation process can be carried out using either a wet or dry method. From an energy standpoint, the dry method has a negative energy balance, due to the high operating costs for dewatering, hence why industrial applications carry out lipid recovery using a wet method (Chisti, 2013; Onumaegbu et al., 2019).

Currently there are several applications of microalgae ranging from biofuel production and CO₂ capture to food supplements and medical applications. The human consumption of microalgae dates back to ancient China where *Nostoc* was used to overcome famine. Other species such as the *Aphanizomenon* and *Arthrospira* were also used to overcome famine (Singh et al., 2005). These health benefits have continued into the 21st century whereby the species *Chlorella* is produced in over 70 countries and is sold as a dietary supplement (Milledge, 2011). The photosynthetic process has made the cultivation of microalgae an excellent process for CO₂ capture. The integration of industrial CO₂ emissions with biorefineries reduces the CO₂ output to the atmosphere while producing greener energy. Large-scale commercial production of biofuel is still being developed, however it is deemed the fuel of the future with current research showing the lipid capacity of algae has the potential for large scale operations...
Besides biofuel, the largest industrial application is within the nutraceutical and pharmaceutical industry. Production of supplements such as the Docosahexaenoic acid (DHA) is estimated to be around 240 tonnes with a net value of approximately $300 million (Spolaore et al., 2006). This single example illustrates the extent microalgae have on the current nutraceutical and pharmaceutical. There is an extensive list of similar chemicals being produced globally with market valuations in the billions of dollars.

There is real potential for what is known as cell factories for the production of other compounds and proteins. According to (Gong et al., 2011) these cultivation factories offer a potentially sustainable large-scale operation through the use of genetic manipulation. Eukaryotic microalgae possess posttranslational modification pathways (Hempel and Maier, 2012). These pathways allow glycosylated proteins to be produced and secreted out of the cell. Only a select few microalgae such as Botryococcus braunii can secrete extracellular biochemicals and they tend to have a longer doubling time which makes them a less suitable candidate for large scale production. However, if secretion of lipids is achieved by genetic modification in rapidly reproducing species then instead of a single use, there can be multiple extractions from the same batch through the process of “milking” (Lauersen et al., 2013). The process of “milking” has the potential to reuse the same batch of microalgae for several extractions (Jackson et al., 2017) which can increase the profitability of each batch significantly.

Cultivation methods can be categorised into two groups: open and closed systems. The current primary cultivation systems for open and closed systems are raceway ponds and photobioreactors, respectively (Barkia et al., 2019). Open pond cultivation is currently the most commonly used in industry due to its low capital and operating costs. However, despite the initial economic benefits, there are significant limitations which Costa and de Morais, discuss extensively (2014). Closed systems offer a more controlled cultivation environment, which have been the main subject for industrial economic biorefinery cultivation technology. (Fan et
al., 2020) determined that the main bottleneck in the industrial application of microalgae was the cost of production (2020), hence why the bulk of research is aiming to reduce the operating cost and improve the production rate/yield. Cultivation optimisation relies on the alteration of operating conditions based on the strand of microalgae being used. Such conditions to control include: temperature, light intensity, light duration, pH, CO₂ concentration and nutrient content (Velichkova et al., 2013; Yoshimura et al., 2013).

Following the cultivation of microalgae, the aim of current research is to extract the high-value compounds within the cells ready for purification. Cell lysis can occur either mechanically or non-mechanically (Günerken et al., 2015). Mechanical extraction relies on the use of force generated by devices such as screw expellers and bead mills (Chisti and Moo-Young, 1986). Non-mechanical extraction involves the use of chemical agents or enzymes which perforate the cell wall/membrane, allowing the intracellular compounds to be leached from the cell (Middelberg, 1995).

Data taken from Walker, Collet and Purton suggests that there are around 40 different species which have been successfully genetically modified in some way which includes C. reinhardtii, Chlorella vulgaris and D. salina. One of the predominant limitations to this process is the low biomass concentration of certain products, which impacts the economic performance of microalgae cultivation. Advancements in algal genome data have been used to enhance the levels of high-value products to overcome the production limitations.

According to Rahman, a concept for analysing the business performance through transforming raw materials into finished products is known as a “value chain” (2020). Relating to microalgae, this chain includes all the main stages within the biorefinery. A market pyramid by Vigani et al., illustrates the volume and value of compounds relating to their respective markets (2015). The nutraceutical and pharmaceutical industry require high value productions
that are produced in low quantities. This is the major bottleneck within these industries, hence more research is needed to improve the output and recovery of these high-value products. Currently *Spirulina* and *Chlorella* are used in the nutraceutical industry due to their production of β-carotene and unsaturated fatty acids (Koyande et al., 2019). However, the cell wall of *Spirulina* is not digestible, which increases the cost of purification. One important research goal is to increase the productivity of these compounds to compensate for the expense incurred for purification. This review aims to review the array of high-value compounds produced by eukaryotic microalgae and discuss the factors influencing their production.

### 2 - Biochemical Properties of Microalgae:

The classification of microalgae has traditionally been set according to their photosynthetic pigments, however, current systems of classification also consider cytological and morphological characteristics (Groendahl et al., 2017). As of 2021, the literature among taxonomists has failed to come to a consensus on a universal classification system apart from the two main groups of eukaryotic and prokaryotic. The key distinction between these two main groups is that eukaryotic cells contain a membrane-bound structure whereas prokaryotic cells do not (Stark and O’Gara, 2012). Through evolution, eukaryotic cells are larger and more complex than prokaryotic cells.

Typically, microalgae species are classified by the type of phyla. The suffix phyla denotes the taxonomic kingdom, and each constituent can be categorised by colour and physical properties. There are approximately seven main phyla, and although they produce almost the same compounds, there are differences in quantity and purity of compounds produced from species to species.
2.1 – Euglenophyta

Euglenophyta are small, unicellular flagellates are predominantly found in freshwater environments and are generally colourless (Gupte and Iyer, 2015). Euglenoid cells do not possess a cell wall, but rather are bounded by a pellicle that can change between plastic and firm characteristics. It is reported by Bloeser, that this interchangeability by the pellicle allows the cells to change shape when in motion (1984).

Like all plants, these algae photosynthesise due to the presence of chlorophyll \( a \) and \( b \) as well as accessory carotenoids. Instead of storing starch, the storage product is the carbohydrate \( \text{paramylum} (\beta_1, 3 \text{ glucose polymer}) \) (Bloeser, 1984). Most species of euglenophyta are known to feed heterotrophically whereby they absorb food through the pellicle boundary via phagocytosis.

According to Bloeser, the reproduction occurs by longitudinal cell division (1984). They are easily distinguished by an eyespot located adjacent to the flagella, which is the collection site of the carotenoid pigments produced by the cell. These cells typically reproduce by asexual longitudinal division with some species forming cysts. The species \( E. \text{viridis} \) is known to produce large toxic blooms in the presence of high nitrogen content (The Editors of Encyclopaedia, 2020).

2.2 – Chrysophyta

Typically, Chrysophyta are referred to as the golden-brown algae that can be unicellular or form complex colonies. In many cases, their cell walls are composed of cellulose, with significant quantities of silica. However, in some species the cells are amoeboid (Myklestad and Granum, 2009). Similar to the Euglenophyta, they contain the photosynthetic pigments chlorophyll \( a \) and \( c \) (Kristiansen and Skaloud, 2016).
According to (Scoble and Cavalier-Smith, 2014) the majority of this species can be found in fresh water, however, research has shown that the *Hebetomonas* and *Acrespina* species can survive and thrive in marine environments (von der Heyden et al., 2004). This species has been reported to reproduce sexually by cell fusion which is followed by encystment of the zygote (Kristiansen and Škaloud, 2016). They produce two endogenous cysts during their life cycle, namely statospores and stomatocycts. In the case of *Dinobryon cylindricum*, encystment occurs during the exponential and stationary phase (Kristiansen and Škaloud, 2017). For sexual cysts to be produced, two clones must be present within the habitat, whereas asexual cysts are produced by individual cells at a low rate during the growth period. This adaptability in reproduction is why this species of microalgae are found on every continent except Antarctica (Nicholls and Wujek, 2003).

**2.3 – Rhodophyta**

Rhodophytes are more commonly referred to as *red algae* due to the presence of the pigment phycobilins. This pigment is also present in the blue-green algae species. These cells contain a double cell wall (Fritsch, 1977), in which the outer layer contains the polysaccharides agarose and agarpectin. The internal cell wall is comprised mainly of cellulose. According to Yoon et al, this species is most commonly found in marine habitats and makes up most of the world's seaweed (2017).

These organisms contain the photosynthetic lamellae pigments, chlorophyll a, $\alpha$-carotene and $\beta$-carotene. These red seaweeds have gained significant interest in the nutraceutical market due to their abundance in several health-promoting molecules such as essential amino acids, omega-3 fatty acids and essential minerals (Cotas et al., 2020). It is suggested by Costas *et al*, that current research should aim to guarantee that Rhodophyta seaweeds produced in
laboratories can produce similar or higher concentrations of these products (2020). Reported
by (Schneider and Wynne, 2013) these cells have been well documented by Bornet and Thuret,
to reproduce sextually and has helped form the basis of classification of the Florideophyceae
(2013).

2.4 – Phaeophyta

Known as the brown algae, Phaeophytans are multicellular organisms which can be found
predominantly in costal environments, often forming dense beds (McCauley and Wehr, 2007).
They form a major component of littoral and sublittoral temperature zones and in subtropical
ecosystems (la Barre et al., 2010). Similar to Chrysohytes, the colour pigmentation is formed
in the cell chloroplast by the presence of chloroylls $a$, $c$ and $c_1$ (Archibald et al., 2017). They
contain a single cell wall comprised of mainly cellulose.

These organisms are well documented to reproduce by a means of fragmentation and sexual
reproduction; however, this can be inhibited by environmental factors such as seasonal
changes, pH, light intensity, and temperature (Mathieson et al., 1981). Phaeophyta have an
essential adaptive feature according to la Barre et al, that utilises oxidative reactions such as
sunlight exposure with the halogenation substrates (2010). This allows the cells to adapt
efficiently to abiotic and biotic stresses.

2.5 – Pyrrophyta

Known as the fire algae for its golden-brown colour, the Pyrrophyta are a class of
Dinoflagellate. Some species are heterotrophic, however, most are photosynthetic organisms
which contain chlorophyll $a$, $c_2$ and $\beta$-carotene. According to a review paper by Hackett et al,
this strand of microalgae has more unique characteristics than other microalgae strands (2004).

Due to their unique pattern of mitosis, their DNA and reproductive systems are significantly different to both eukaryotic and prokaryotic cells. Pyrrophyta are well-established as primary producers and consumers within the food web. It is not uncommon for these algae to consume their own metabolites freely with their own ecosystem. This behaviour is usually only observed when a lack of nutrients develops with a system.

It was proposed by Spector, that a new kingdom known as Mesokaryota should be established (1984). Many species are known to produce significant neurotoxins, which produce the phenomena known as red tides. Due to the large quantities of pigments, the toxic blooms can stain the waters and surrounding beach red, which are reported by Smayda, to be non-toxic (1997). Research has found that these blooms provide essential nutrients to corals and marine invertebrates that help support their ecosystem (Cotas et al., 2020). However, a paper published by (Lehane and Lewis, 2000) showed that there had been several cases where humans had been poisoned and killed by the blooms, both directly and indirectly.

2.6 – Xanthophyta

Xanthophytans, also known as the yellow-green algae, are most commonly found in freshwater habitats. They are generally unicellular or coenocytic which are only a few millimetres long. This class was once considered the same as the green Chlorophyta algae on the basis of colonial organisation and body similarities; however, it was found that xanthophyta were closely genetically related to Phaeophyta, resulting in a new class of algae. According to Maistro et al, species such as Vaucheria form thick velvet-like mats that allow them to bind sediment in salt marshes, allowing them to regulate their ecosystem and distribute minerals (2016).
According to Kumar and Singh, these cells aren’t as evolved as the green algae *chlorophyta*, therefore they lack the advanced elaborate pseudoparenchymatous habits (1979). They contain, like most microalgae, the pigments chlorophyll *a*, *e* and β-carotene; the latter found in high concentrations. They can reproduce both sexually and asexually, however, sexual reproduction is rare and has only been observed in three genera: *Vaucheria* (Kumar and Singh, 1979; Maistro et al., 2016)

### 2.7 – Chlorophyta

Chlorophytans are highly diverse and specialised cells known as the *green algae*, and can be found in most habitats around the world. Mostly aquatic however, they are known to be found in moist soil and even in snowbanks according to Leya (2013). They vary in size and complexity from unicellular to multicellular and coenocytic (more than one nucleus per cell) colonies. According to Fang *et al*, chlorophyta the evolution of this class can be traced to over 1 billion years ago, which illustrates why this strand of microalgae are regarded as the most advanced (2017). They are known to form on the leaves terrestrial plants and are considered to be parasitic (Gangadhar et al., 2019).

Due to the diversity of this group, reproduction can occur both sexually and asexually. For the strand *Botryococcus braunii*, cellular reproduction occurs by autosporic mechanisms, whereas asexual reproduction can occur in the strand *Chlorella vulgaris*, which involves 2 to 4 daughter cells according to Yamamoto *et al.*, (2003).
2.8 – Biochemical Composition

The biochemical composition of microalgae’s biomass comprises of protein, carbohydrate, and lipids (Jackson et al., 2017). It is important to understand the biochemical composition of microalgae before considering the extraction and purification of other high-value products. It should be noted that the data shown in Table 1 does not account for different sub-species, but rather an average w/w percentage of each compound, as some report suggest that the strand *Botryococcus braunii* can have a lipid content of 80% (Velichkova et al., 2013).

A comparative illustration of the variation of Chlorophyta species is shown in Figure 1 (Hallmann, 2019). The diversity of shape, pigment and taxonomy of these species shows the evolution of the eukaryotic cells.

*Figure 1. Variation of Chlorophyta Species* (Velichkova et al., 2013)
Table 1. Products from eukaryotic microalgae

<table>
<thead>
<tr>
<th>Species</th>
<th>Protein (w/w)</th>
<th>Carbohydrates (w/w)</th>
<th>Lipids (w/w)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Botryococcus braunii</em></td>
<td>40%</td>
<td>6%</td>
<td>33%</td>
<td>(Metzger and Largeau, 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Babu and Wu, 2008; Cheng et al., 2019)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Babu and Wu, 2008)</td>
</tr>
<tr>
<td><em>Chlorella luteoviridis</em></td>
<td>47%</td>
<td>12%</td>
<td>22%</td>
<td>(José de Andrade and Maria de Andrade, 2017)</td>
</tr>
<tr>
<td><em>Chlorella sorokiniana</em></td>
<td>56%</td>
<td>17%</td>
<td>22%</td>
<td>(Chai et al., 2018)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(Lizzul et al., 2018)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(Lammers et al., 2017)</td>
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<td></td>
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<td></td>
<td></td>
<td>(León-Vaz et al., 2019)</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>68%</td>
<td>9%</td>
<td>26%</td>
<td>(Lorenz and Cysewski, 2000)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(Boussiba, 2000)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(Olaizola, 2000)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Haque et al., 2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Ding et al., 2019)</td>
</tr>
<tr>
<td><em>Parachlorella kessleri</em></td>
<td>51%</td>
<td>16%</td>
<td>25%</td>
<td>(Li et al., 2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Qu et al., 2019)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Sharma et al., 2019)</td>
</tr>
<tr>
<td><em>Scenedesmus subspicatus</em></td>
<td>28%</td>
<td>29%</td>
<td>16%</td>
<td>(Dantas et al., 2019)</td>
</tr>
<tr>
<td><em>Chlorella pyrenoidosa</em></td>
<td>57%</td>
<td>26%</td>
<td>2%</td>
<td>(Chisti, 2007)</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>58%</td>
<td>17%</td>
<td>22%</td>
<td>(Becker, 2007)</td>
</tr>
<tr>
<td><em>Scenedesmus obliquus</em></td>
<td>56%</td>
<td>17%</td>
<td>14%</td>
<td>(Becker, 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(González López et al., 2010)</td>
</tr>
<tr>
<td><em>Dunaliella salina</em></td>
<td>57%</td>
<td>32%</td>
<td>6%</td>
<td>(Becker, 2007)</td>
</tr>
<tr>
<td><em>Chlamydomonas reinhardtii</em></td>
<td>48%</td>
<td>17%</td>
<td>21%</td>
<td>(Becker, 2007)</td>
</tr>
</tbody>
</table>
2.8.1 – Proteins

Proteins are formed from the fundamental biological building blocks – amino acids – which are essential for all life on Earth. According to Papadopoulos, the production of amino acids might be a by-product of the algal process since the free amino acid composition varies greatly by species and growth conditions (2008a). Certain species of *Chlorella vulgaris* are said to have a 58% protein on a dry basis (Becker, 2007). Microalgae are often considered as an unconventional protein source according to Soletto et al, (2005). Ancient Aztec cultures have been consuming microalgae as a source of protein for thousands of years (García et al., 2017). It is worth noting that the application of microalgae proteins has been limited in the food industry due to the presence of high concentrate non-protein compounds such as chlorophyll (Becker, 2007). On the contrary, if cells have a low cellulosic concentration, they have a better digestibility for human consumption (Cohen and Vonshak, 1991).

2.8.2 – Carbohydrates

Carbohydrates are complex organic compounds formed inside the chloroplast as a product of photosynthesis. In eukaryotic organisms, starch is synthesized in the form of two polysaccharide glucose polymers: amylose and amylopectin (Busi et al., 2014). While the carbohydrates have the lowest energy content compared to lipids and proteins, they serve important functions for structural features of the cell wall and energy storage (Barkia et al., 2019).

Amylose is less readily digested than amylopectin due to its helical structure, which takes up less space and accounts for approximately 30% of the stored starch (Wang et al., 2017). According to Dismukes et al, this storage capacity can increase to 80% dry weight during the exponential growth phase, under nutrient starvations and stress conditions (2008). This claim
is also confirmed by Markou et al., (2012). While carbohydrates from microalgae are used in
the food industry, their applications are limited. While lipids are the main component to
biofuels, it was reported by Markou et al, that carbohydrates are the main substrate for biofuel
production (2012). Their use has also gained popularity in the cosmetics industry as
antioxidants and topical creams (de Jesus Raposo et al., 2013).

2.8.3 – Lipids

The most widely researched and commercialised biochemical product is lipids A paper
published by Gopal Satpati et al, determined the lipid composition of twenty-one stands of
microalgae taken from the Sundarban area in India; six of which were freshwater (2012). The
saturated, mono-unsaturated, and poly-unsaturated fatty acids were measured and summarised
in Table 2 for several species of freshwater microalgae (Gopal Satpati et al., 2012; Nascimento
et al., 2013).

Lipids can be broken down into saturated and unsaturated fats. Saturated fats are usually in the
form of neutral lipids such as free fatty acids and carotenoids (Williams and Laurens, 2010).
Unsaturated fats are usually in the form of polar lipids such as phospholipids and galactolipids.
Their main function is to maintain membrane fluidity under cultivation conditions (Williams
and Laurens, 2010).

While there are other uses of lipids in the form of fatty acids in nutraceuticals and cosmetics,
biodiesel is dominant use of lipids in the C16 to C18 carbon length chain molecules (Dunstan
et al., 1993). Lipids help form part of the plasma membrane and act as secondary energy
sources (Barkia et al., 2019).

Much research has been undertaken to determine the optimal growth conditions to maximise
the production of lipids. The production rate is dependent on the species, cultivation conditions,
and extraction method. For example, the species Botryococcus braunii has a lipid content of 33% according to Metzger and Largeau, (2005). However, according to Jackson et al, the same species can have a lipid content of 80% on a dry weight basis (2017). Both claims are backed by empirical evidence, which shows how the cultivation conditions can have a significant impact on the lipid productivity. Many studies have shown increased productivity in the exponential growth phase when stress is applied (Yousuf, 2019). Some species such as the B. braunii secrete lipids during its lifecycle; little is known as to why some species secrete extracellular lipids, however, one theory suggests it is for buoyancy (Jackson et al., 2017).

According to Niehaus et al, the buoyancy is used as a mechanism to float within the normal aquatic habitat that allows the cells to maximise the surface area exposed to light, resulting in an increased photosynthetic rate and an improved metabolic performance (2012).

Research conducted by (Onumaegbu et al., 2019) looked at the microwave pre-treatment of Scenesdemus quadricauda (2019). This species has been shown to have a 77% lipid content on a dry basis (Balasubramanian et al., 2011). Onumaegbu et al, considered using microwaves to disrupt the cells in order to extract the lipids. They concluded that this method of extraction using 600W of microwaves for up-to 8 minutes on a wet-basis enhanced the lipid recovery to 49%. This is significantly higher than previous research conducted by Cheng et al, achieved a 38.46% lipid extraction on a dry basis (2014). It was concluded that using wet biomass has a higher lipid yield for improved economic biodiesel production (Onumaegbu et al., 2019).

A similar, alternative method to microwave extraction is ultrasonication, which induces shear forces that cause cavitation within the cell walls (Mendes-Pinto et al., 2001). A study by Halim et al, determined that microwave extraction was more effective as the operating power of up-to 130W for ultrasonication had no significant effect on rupturing the robust cell wall (2012).

More traditional methods of lipid extraction combine the effects of cell lysis and lipid recovery. They involve the single use of the microalgae whereby the cell wall is permanently ruptured to
allow the intracellular lipids to be recovered. Such systems include solvent extraction which
the cells are exposed to an organic solvent, typically hexane, acetone, and chloroform (Lehr
and Posten, 2009). Regarded as the first documented method of solvent extraction, the Folch
method is still used in selected processes. However, due to the chloroform and methanol
solvents used, more environmentally friendly systems are being developed such as ionic liquid
extraction (Ranjith Kumar et al., 2015).

Transfection is the process by which nucleic acid molecules, such as DNA, can be introduced
into a culture of eukaryotic cells (Hoffmann et al., 2019). This can be achieved by viral
transfection and non-viral transfection. Non-viral chemical transfection relies on the
electrostatic interactions between the reagents and the cell membranes. Typically, DNA is
negatively charged, which is repelled by the negatively charged cell membrane, making the
transfer of material difficult. Liposome-mediated transfection refers to the use of cationic lipids
such as N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride to carry the nucleic
acid molecules through the cellular membrane (Felgner et al., 1987). Once across the cell
membrane, the reagent releases the nucleotides into the cytoplasm via endocytosis (Hoffmann
et al., 2019). This is a cost effective, efficient, and highly reproducible system for genetic
research.
Table 2 – Fatty acid profile of freshwater microalgae (Nascimento et al., 2013) (Gopal Satpati et al., 2012).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Synechocystis pevalekii</th>
<th>Nostoc ellipsoспорum</th>
<th>Spirulina platensis</th>
<th>Spirogyra orientalis</th>
<th>Chlorococcum infusionum</th>
<th>Rhizoclonium fontinales</th>
<th>Botryococcus braunii</th>
<th>Chlorella vulgaris</th>
<th>Pseudokirchneriea subcapitata</th>
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<tbody>
<tr>
<td>Total Lipids in Biomass</td>
<td>9.00</td>
<td>7.00</td>
<td>8.50</td>
<td>21.00</td>
<td>11.34</td>
<td>7.50</td>
<td>44.97</td>
<td>28.07</td>
<td>28.43</td>
</tr>
<tr>
<td>(% dry weight)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Saturated Fatty Acids (SFA)</td>
<td>36.80</td>
<td>39.25</td>
<td>51.03</td>
<td>38.98</td>
<td>42.43</td>
<td>40.83</td>
<td>9.85</td>
<td>52.15</td>
<td>35.39</td>
</tr>
<tr>
<td>(% dry weight)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mono-Unsaturated Fatty Acids (MUFA)</td>
<td>33.60</td>
<td>30.15</td>
<td>21.12</td>
<td>8.22</td>
<td>21.12</td>
<td>28.01</td>
<td>79.61</td>
<td>37.51</td>
<td>47.36</td>
</tr>
<tr>
<td>(% dry weight)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyunsaturated Fatty Acids (PUFA)</td>
<td>29.60</td>
<td>30.60</td>
<td>27.85</td>
<td>52.80</td>
<td>36.45</td>
<td>31.16</td>
<td>10.54</td>
<td>10.33</td>
<td>17.25</td>
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</tbody>
</table>
3 – High-Value Compounds

The biochemical composition of microalgae’s biomass comprises of proteins, carbohydrates, polyunsaturated fatty acids (PUFA), pigments, polysaccharides, phytosterols, and vitamins (Papadopoulos, 2008b). Microalgae also synthesise several secondary metabolites with a multitude of uses. These metabolites have been found to act as antioxidants, anticancer properties, anti-inflammatory properties and much more. According to (Botana and Alfonso, 2015) these bioactive compounds are produced through the shikimic acid, mevalonic/non-mevalonic acid pathways. It is believed that there are thousands of bioactive compounds produced by eukaryotic microalgae (Manning and Nobles, 2017). There are approximately anywhere between 200,000 to 600,000 different species of eukaryotic microalgae (Esteves et al., 2020), however, most haven’t been studied extensively for their commercial potential.

Evolution has contributed to the diverse applications of eukaryotic microalgae since they are one of the earliest forms of life. Their high-value compound category is fairly broad with applications in the cosmetics, food formulation, animal feed stock, wastewater treatment, health products and biofuel production. However, to-date most of the research has failed to scale up-to commercial production due to several reasons, namely high operating costs, market competitiveness and strict regulations. It is clear that the predominant industry for microalgae production is in the pharmaceutical and nutraceuticals due to the rise in public perception of synthetic chemicals and nutritional benefits supplements have on the human body. This shift to natural products has seen the utilisation of high-value products from microalgae a lucrative industry.

Referring to the value chain and market pyramid discussed in section 1, the nutraceutical and pharmaceutical industries require the highest value compounds that are produced in the lowest quantities such as carotenoids, vitamins, and antioxidants. To improve the selectivity and
productivity of these compounds, research into the factors influencing the growth rate and compound production is essential to commercialising microalgae biorefineries. When conditions such as temperature, pH, salinity, nitrate concentration etc are manipulated they induce stress conditions. These stresses cause the microalgae to react and over produce compounds that will regulate their environment (Minhas et al., 2016).

Traditionally, the use of microalgae was for a one-product purpose, usually for lipid extraction only. After the desired product was obtained the spent algae would be discarded. The initial optimisation for the system saw the spent algae dried and used as fuel for furnaces. Now, the biorefinery is a much more sophisticated system – similar to a petroleum refinery. A typical biorefinery output is shown in Figure 2 (Postma et al., 2017).
3.1 – Carotenoids

According to Levasseur et al. carotenoids account for 0.1 to 0.2% of the dry matter produced by microalgae (2020). These high-value compounds play a vital role in the photosynthesis and provide photoprotection of high light exposure (Novoveská et al., 2019). It has been reported Novoveská et al., that to increase the production of carotenoids such as β-carotene, high light intensity and higher concentration of nitrates are required (2019). The increased light generates excess energy production within the chlorophyll, and this stress inducing condition forces a higher production of carotenoids to remove and dissipate this surplus energy (Novoveská et al., 2019). This methodology is at the forefront or research into increasing the selectivity of all high-value compounds from a wide range of microalgae species.

Carotenoids act as accessory colour pigments found in photosynthetic organisms. They also play a vital role in protecting the cells from free radical attack, whereby they quench a single oxygen and trapperoxyl radical (Skibsted, 2012). The more traditional use of carotenoids was for natural food colourings for chicken and fish. They have also been used in the cosmetics industry for their antioxidant properties and natural colourings. According to Santhosh et al. there are approximately 750 different carotenoids, with only a select few being commercialised such as β-carotene, astaxanthin and lutein (2016). Most carotenoids have a similar chemical structure and are composed of an 18-carbon conjugated double bond which has two hexacarbon rings at each end (Odjadjare et al., 2017). The synthesis of these biproducts has traditionally been carried out by regular photosynthesis however, a paper published by Li et al, has successfully designed a hybrid cell factory which uses gold nanoparticles to enhance the photosynthetic rate and efficiency to increase carotenoid production (2020). Figure 3 illustrates the process designed by (Li et al., 2020).
The results showed that there was an increase in carotenoid production to 10.7 ± 1.2 mg/L, which is a 42.7% increase than natural photosynthesis. Li et al., concludes that the increase in production is due to the significant improvement in the relative electron transport rate within the photosystem, resulting in a more reactive oxygen species.

### 3.1.1 – β-carotene:

β-carotene was the first high-value compound to be commercialised according to (Rammuni et al., 2019). It is one of the most commonly produced carotenoids across most species of microalgae, however, the microalgae *Dunaliella salina* has been reported to have a 98.5% of its total carotenoids comprised of β-carotene, accounting for around 13% of its dry mass (Molino et al., 2018a). β-carotene has unique properties that inhibit the growth of cancer cells by inducing apoptosis in a wide range of cancer cells (Cooper, 2004). Due to the rise in public perception of the adverse effects of drug therapy, the public’s focus has shifted towards natural
health-promoting products; hence there is a growing demand for the β-carotene in nutraceuticals and pharmaceuticals (Rammuni et al., 2019).

3.1.2 – Astaxanthin:

Following β-carotene, astaxanthin is the second most commercialised carotenoid. Known for its distinctive red pigment (xanthophyll), it is mainly used in aquaculture as a food dye (Bhalamurugan et al., 2018), traditionally for salmon, trout, lobster, and shrimps. From a nutritional perspective, astaxanthin is referred to as the most powerful antioxidant in nature (Panis and Carreon, 2016) (Koller et al., 2014), due to its ability to scavenge for free radicals quickly and efficiently. This property is considerably useful for skin protection against UV-induced photo-oxidation and helps delay the ameliorating age-related disease and has been referred to as the fountain of youth (Chue et al., 2012). While many species of algae produce astaxanthin, the species Haematococcus pluvialis can accumulate up-to 81% of its total carotenoids with a corresponding dry weight of 7% according to (Molino et al., 2018b). Over recent years astaxanthin has become widely used in cancer treatment, ocular health, diabetes and anti-inflammatory disease treatment (Hussein et al., 2006). More recent studies have discovered that it has a particularly positive impact on the prevention and cure of colon cancer (Nagendraprabhu and Sudhandiran, 2011).

3.1.3 – Lutein:

The yellow carotenoid lutein is mainly used in the drug and cosmetic industry. According to Roberts and Dennison, lutein can help protect against damage to the retina and lens of the eye (2015). These antioxidants accumulate in the eye and prevent peroxide damage from singlet oxygen (Edge et al., 1997). While these powerful antioxidants are useful in cosmetics and
pharmaceuticals, the production capacity of microalgae is only around 0.5% to 1.2% according to Levasseur et al., (2020). Currently, marigold flowers have around 20g/kg of lutein (Bhalamurugan et al., 2018), making them the primary producer of lutein. Levasseur et al. discusses that a production of 0.5% lutein on a dry basis has a long way before it can be commercialised (2020). This is contradictory of Molino et al. who states that 1.2% on a dry basis is considerably higher than conventional sources (2020). From current research, the lutein from flowers is the most accepted and widely commercialised system. Lutein has recently been found to prevent cardiovascular diseases (Coleman and Chew, 2007).

3.2 – Polyunsaturated Fatty Acids

Polyunsaturated Fatty Acids (PUFA’s) are long carbon chained molecules with multiple double bonds and are part of the lipid family. These hydrocarbon organic molecules are usually contained to the phylum in all eukaryotic microalgae (Lang et al., 2011; Sahu et al., 2013). Microalgae have been referred as the richest source of PUFAs according to Ramesh Kumar et al., (2019). These high-value products are applicable to the food and pharmaceutical industry. However, from a medical perspective, PUFAs offer significant health benefits to a wide range of chronic conditions. Such conditions include diabetes, arthritis, obesity, inflammatory conditions, and more recently cardiovascular disease (e.g., hypertension, myocardial infraction, and cardiac arrhythmia) (Adarme-Vega et al., 2012; Hamed, 2016)

A study carried out by (Sahu et al., 2013) determined the fatty acid profile of 12 strains of microalgae and determined that the Chlorophyceaeas were rich in palmitic acid (C16), oleic acid (C18) and linoleic acid (C18) (2013). However, in terms of nutrition and medicinal purposes microalgae typically produce two main types of essential fatty acids – Docosahexaenoic acid
(DHA) and Eicosapentaenoic acid (EPA) (Adarme-Vega et al., 2012). They are part of the essential *Omega fatty acid* (ω-3).

It is reported that regular consumption of these essential fatty acids as supplements has been shown to reduce and even prevent cardiovascular disease (Adarme-Vega et al., 2012). Similar health benefits reported by (Santhosh et al., 2016) showed that linoleic acid has been used to treat skin hyperplasias. However, it was reported by Odjadjare et al. that due to the poor oxidative stability, toxins can be produced which restrict the potential applications due to the unpleasant smells released (2017). The main sources of these essential fatty acids are found in fatty fish such as salmon.

Current research has shown that DHA is the main PUFA product on the market, however, there are three main strategies outlined by Chauton et al. which aim to increase the productivity of PUFAs (2015). The first aims to regulate the cultivation conditions using the same techniques for increased lipid production. Secondly, a selective breeding of different species can improve the lipid productivity. Last, genetic engineering techniques are being applied to target the production of DHA and EPA. At present the only quantifiable production of these PUFAs can be seen in the *Chlorella* species with experimental reports claiming production levels of DHA and EPA to be 3.24% and 8.9% respectively (Wang et al., 2019).

Based on the literature, the proposed condition improvement scenario favours the lipid production, with a trade-off with a reduced overall growth rate. There are several methods used to analyse fatty acids produced by microalgae, including the traditional Bligh and Dryer method to ionic liquids and sonication (Li et al., 2014). According to Li et al. the SC-CO2 method yielded the values of fatty acids however, subsequent methods such as solid-phase extraction columns offer a higher accuracy for lipid recovery and quantification (2014).
3.3 – Phytosterols

Phytosterols are a sub-group of steroids sometimes referred to as sterols. They have a significant use in the pharmaceutical industry with applications in cholesterol-lowering, anti-inflammatory, antioxidant, and anti-cancer treatment (Luo et al., 2015). With the growing importance of natural skincare products, sterols are being used to improve skin hydration and protection against free radicals (Spanova and Daum, 2011). Currently the main sources of phytosterols are vegetable and tall oils (Randhir et al., 2020) with an estimated market value of $490 million by 2022. While the production of phytosterols from vegetable oils is a cost-effective and well-established industry, there is growing concerns for the sustainability of the current production methods which require substantial areas of land.

A paper published by (Volkman, 2016) provides extensive biochemical research into the diversity and biosynthesis of phytosterols produced by microalgae. The research is still in its infancy, and as a result there is little published data on which strands produce the greatest quantities and types of phytosterols. However, it has been reported by Rampen et al. that the diversity of sterols in microalgae is a hallmark of diatoms and showed that out of 44 sterols detected 11 of them were commonly present in most microalgae species (2010). Hence with further research and development microalgae could surpass the current production rate of phytosterols from vegetable oils.

Structurally, sterols consist of a cyclepentaphenanthrene ring and a side chain at the C-17 carbon. Most microalgae sterols have been identified and quantified by GC-MS analysis (Randhir et al., 2020). This method has its limitations in separating the stereoisomers and as result, nuclear magnetic resonance (NMR) has been proposed to measure the different sterols.

3.4 – Polyphenols
Polyphenols are polymers of phenolic compounds which are part of a diverse group of secondary metabolites containing at least one hydroxyl group attached to an aromatic ring. These molecules share similar properties to phytosterols, however, they are particularly effective in radical scavenging. Due to their variety of biological properties, the polyphenols have a profound application in the pharmaceutical and nutraceutical industries. While oxygen is essential for life, it can have a negative impact on living organisms by inducing oxidative stress in cells, which is a major cause for several cancers (Sansone and Brunet, 2019). These molecules provide biological antioxidants which are of particular interest in anti-cancer, anti-diabetes, and anti-inflammatory research (Galasso et al., 2019).

Polyphenols as well as other high-value compounds from microalgae are strong oxidising agents whereby they have the ability to provide electrons to highly energetic free radical particles which terminate the destructive nature of free radical particles. They play a vital role in the prevention of degenerative neuropathies and cardiovascular diseases (Wang et al., 2018).
Polyphenols form several other polymers including, flavonoids, flavanols and tannins. Each polymer can form several variations which have important applications in the food and pharmaceutical industries. Figure 4 shows a flowchart of the different subdivisions of polyphenols (Zhang and Tsao, 2016).

3.5 – Vitamins

Microalgae act as a primary food source in aquatic habitats and are responsible for the transfer of essential vitamins and minerals along food chains. Since humans and other animals are not able to synthesise their own essential vitamins and minerals, the only means of obtaining them is through direct digestion of the raw microalgae or in the form of supplements. The nutraceutical industry is dependent on the productivity and diverse range of high-value
products from microalgae. Aquatic microalgae produce and accumulate a wide range of vitamins including, vitamin A, B, C, D and E (Galasso et al., 2019).

There is extensive literature published on the dependency of the growth conditions on the content and quality of the vitamins produced by microalgae. There is significant overlap in the growth conditions and optimisation for vitamins, carotenoids, and lipids (Toti et al., 2018). A study by Brown and Farmer, illustrated that the production of vitamin B2 in six microalgae species increased during the beginning of the stationary growth phase (194). Vitamins C and E have been reported by Barbosa et al. to be at their highest during the exponential growth phase, while vitamins A and B were at their highest levels during the stationary phase (2005). From the literature it became clear the interactions between optimal growth conditions and production of vitamins varied significantly between each species. However, within a single species the production of vitamins can also vary. A study by Barbosa et al. found that the relationship between light availability and vitamin yield is the Dunaliella tertiolecta species had an increase in vitamin C content from 1.72 to 3.48 mg/g, whereas the amount of vitamin E produced decreased significantly (2005). Table 3 summarises the vitamin content of several species of Chlorophyta eukaryotic microalgae which illustrates the variation and presence of each vitamin on a mg/g dry weight basis.
Table 3. Vitamin content of eukaryotic microalgae

<table>
<thead>
<tr>
<th>Species</th>
<th>Vitamin A (mg/g DW)</th>
<th>Vitamin B1 (mg/g DW)</th>
<th>Vitamin C (mg/g DW)</th>
<th>Vitamin E (mg/g DW)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydomonas</td>
<td>0.11-0.13</td>
<td>-</td>
<td>2</td>
<td>0.34-4</td>
<td>(Aaronson et al., 1977) (Mudimu et al., 2017)</td>
</tr>
<tr>
<td>Chlorella</td>
<td>0.01-0.65</td>
<td>18-23</td>
<td>0.1-15</td>
<td>0.01-4</td>
<td>(Edelmann et al., 2019) (Woortman et al., 2020) (Aaronson et al., 1977) (Fabregas and Herrero, 1990) (Mudimu et al., 2017)</td>
</tr>
<tr>
<td>Dunaliella</td>
<td>0.01-0.63</td>
<td>9-29</td>
<td>0.16-2.2</td>
<td>0.12-1.9</td>
<td>(Woortman et al., 2020) (Fabregas and Herrero, 1990)</td>
</tr>
<tr>
<td>Haematococcus</td>
<td>-</td>
<td>4.7</td>
<td>-</td>
<td>0.27-0.88</td>
<td>(Mudimu et al., 2017)</td>
</tr>
<tr>
<td>Scenedesmus</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>0.08-1</td>
<td>(Aaronson et al., 1977) (Mudimu et al., 2017)</td>
</tr>
<tr>
<td>Stichococcus</td>
<td>0.06</td>
<td>29</td>
<td>2.5</td>
<td>0.13-0.44</td>
<td>(Brown et al., 1999) (Mudimu et al., 2017)</td>
</tr>
<tr>
<td>Tetraselmis</td>
<td>0.05-4.28</td>
<td>-</td>
<td>0.19-3</td>
<td>0.04-6.32</td>
<td>(Fabregas and Herrero, 1990) (Brown et al., 1999) (Mudimu et al., 2017)</td>
</tr>
</tbody>
</table>

It was clear from the findings of several research papers that the *Chlorella* and *Dunaliella* species were the most diverse in vitamin production. Based on these findings, it was reported that the dry weight content of *Tetraselmis* has a higher vitamin A content than raw carrots (0.011 mg/g DW) (Beltrán-de-Miguel et al., 2015). The species *Chlorella* had a higher vitamin C content than strawberries and lemons (0.54 and 0.42 mg/g DW respectively) (Szeto et al., 2002). It is clear that no one species of microalgae can adequately produce enough of each vitamin to make it economically viable; therefore, the conditions should be tailored to suit the production of a single type of vitamin. Table 4 summarises the high-value products of several species with their respective application.
Peptides derived from microalgae have been shown to be effective alternatives within the pharmaceutical and the food industry (Vo et al., 2013). Regarding pharmaceutical applications, the peptides used are derived from the enzymatic hydrolysis of proteins, usually around 2 to 30 amino acid length (Hayes et al., 2017). The peptides are initially inactive whilst being part of the parent protein. According to Hayes et al. the action of the proteolytic enzymes releases the peptide from the parent protein which results in the activation (2017).

One of the most commercially used peptide producers is the *Chlorella sp* microalgae strand (Ejike et al., 2017). Their bioactive compounds have gained significant attention due to their inherent safety within the pharmaceutical industry, specifically in the applications of anti-inflammatory, anticancer, antioxidant and general human health improvements (Galasso et al., 2019) (Sabzi et al., 2021). According to Hayes et al. one of the key health benefits of microalgae derived peptides is the cardiovascular system, whereby they inhibit enzymes within the renin-angiotensin-aldosterone system which results in a reduction in blood pressure (2017).

One of the limitations on the extraction of peptides is during the hydrolysis the microorganisms can consume the peptides and amino acids as their own substrates according to Nasri, resulting in a poor yield (2017). A method proposed by Maehre et al. using the thermal denaturing of *Palmaria palmate* was reported to improve the yield of peptides from 64% to 96% (2016). While Maehre *et al*, doesn’t offer an explanation for these findings, a report by (Amaya-Farfan, 2021) discusses the chemical changes that occur during the heating process (2021). Such changes include the cleavage of the peptide bonds and chemical alterations that make them undigestible by the microalgae. This therefore transcribes as an improved yield when compared to non-thermal denatured systems.
### Table 4. High value compounds from eukaryotic microalgae

<table>
<thead>
<tr>
<th>Species</th>
<th>Pigment Composition</th>
<th>Fatty Acid Composition</th>
<th>Applications</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Botryococcus braunii</em></td>
<td>6% α-carotene 6% β-carotene 22% lutein</td>
<td>C16:0 29.5%</td>
<td>Biofuels</td>
<td>(Metzger and Largeau, 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C16:1 2.3%</td>
<td>Food additives</td>
<td>(Babu and Wu, 2008; Cheng et al., 2019)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C18:0 1.0%</td>
<td>Pharmaceuticals</td>
<td>(Babu and Wu, 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C18:1 44.9%</td>
<td>Cosmetics</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C18:2 21.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other: 0.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chlorella luteoviridis</em></td>
<td>Total chlorophyll: 29.8 mg/g Total carotenoid: 3.4 mg/g</td>
<td>C14:0 2.4%</td>
<td>Biofuels</td>
<td>(José de Andrade and Maria de Andrade, 2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C16:0 25.0%</td>
<td>Animal Feed</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C16:1 9.3%</td>
<td>Nutraceuticals</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C18:0 7.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C18:1 21.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C18:2 9.7%</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>C18:3 24.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other: 0.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chlorella sorokiniana</em></td>
<td>Total chlorophyll: 32.4 mg/g 1.2 mg/g β-carotene 7.1 mg/g lutein</td>
<td>C16:0 22.0%</td>
<td>Biofuel</td>
<td>(Chai et al., 2018)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C16:1 4.3%</td>
<td>Animal Feed</td>
<td>(Lizzul et al., 2018)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C16:2 11.5%</td>
<td>Nutraceuticals</td>
<td>(Lammers et al., 2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C16:3 5.1%</td>
<td></td>
<td>(León-Vaz et al., 2019)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C18:0 3.5%</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>C18:1 11.3%</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>C18:2 31.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C18:3 9.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other: 2.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>23.2 mg/g Astaxanthin 2.8 mg/g β-carotene 10.2 mg/g lutein 5.8 mg/g chlorophyll</td>
<td>C16:0 22.4%</td>
<td>Nutraceuticals</td>
<td>(Lorenz and Cysewski, 2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C16:1 0.6%</td>
<td>Cosmetics</td>
<td>(Boussiba, 2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C16:2 2.1%</td>
<td>Pharmaceuticals</td>
<td>(Olaizola, 2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C16:3 3.1%</td>
<td></td>
<td>(Haque et al., 2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C16:4 5.8%</td>
<td></td>
<td>(Ding et al., 2019)</td>
</tr>
<tr>
<td></td>
<td>Fatty Acid Composition</td>
<td>Biofuels</td>
<td>Animal Feed</td>
<td>Nutraceuticals</td>
</tr>
<tr>
<td>--------------------</td>
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<td>----------------</td>
</tr>
<tr>
<td><strong>Parachlorella kessleri</strong></td>
<td>C18:0 0.9% C18:1 19.5% C18:2 28.7% C18:3 12.6% Other: 4.3%</td>
<td>Biofuel</td>
<td>Animal Feed</td>
<td>Nutraceuticals</td>
</tr>
<tr>
<td></td>
<td>23.6 mg/g chlorophyll 4.1 mg/g total carotenoid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Scenedesmus subspicatus</strong></td>
<td>C14:0 1.1% C16:0 12.1% C16:1 7.2% C18:0 4.2% C18:1 24.2% C18:2 23.5% C18:3 26.8% C20:0 0.5% Other: 2.1%</td>
<td>Biofuel</td>
<td>Animal Feed</td>
<td>Nutraceuticals</td>
</tr>
<tr>
<td></td>
<td>19.6 mg/g chlorophyll 0.3 mg/g total carotenoid</td>
<td></td>
<td></td>
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</tbody>
</table>
4 – Other High-Value Products

Microalgae are well known for their polysaccharide production, and other high-value products. One variant of the polysaccharide family is the sulphated polysaccharide (sPS); these have been shown to possess the same antioxidant and anti-inflammatory effects as their counterparts. However, not many studies have considered these high-value products yet. Few studies have reported the detailed extraction mechanism for sPS; one such paper was published by Chen et al. which showed that there was significant immunostimulatory effects on the RAW 264.7 murine macrophage cells which is categorised by the up-regulation of interleukin-6 (2019). This shows the significance of such compounds and is one that should be explored further in the extraction of microalgae.

It is generally reported throughout the literature that microalgae have the potential to produce other biochemical compounds, however, the actual clarification of such compounds is not well documented. As far as other bioactive compounds are concerned most literature discusses the potential applications rather than the compounds themselves (Sun et al., 2018). One such paper that does discuss other metabolites is (Barkia et al., 2019). The mention of the sulphated polysaccharides, peptides and phenolics coincides with other literature based on their applications. It is clear that bioactive compounds are of particular interest to the pharmaceutical and nutraceutical industry due to their powerful antioxidant properties.

One such application is the use of these secondary metabolites in immunotherapy (Sun et al., 2018). This relatively new treatment in the fight against cancer has shown significant results in removing the cancerous cells while lowering the severity of the side effects of current cancer treatments. The activation of the cytotoxic T cells by the bioactive compounds from microalgae use the body’s own immune system to attack the cancer cells. This type of innovation is being replicated in other fields of medicine and pharmaceuticals (Hirano et al., 2006).
5 – Discussion

Microalgae are one of the most diverse species on the plant; having several high-value products which are applicable to a wide range of industries. Traditionally, it was thought that microalgae had three products; lipids, proteins, and carbohydrates, however, over the past few decades research has shown there are other high-value products which can be refined and used in several different industries. The focal point of this review paper was the freshwater eukaryotic microalgae to illustrate their importance tackling climate change and providing natural alternatives in cosmetics and pharmaceuticals. The biodiversity of eukaryotes has made them appealing for microalgae research, however, the abundance of different species adds significant complications when researching behavioural and biochemical responses to stress conditions.

The biorefinery concept will ultimately be driven by market demand. This puts significant emphasis on converting microalgae from a single niche to a wider and varied market. From the literature, it is clear to assume that the nutraceutical and pharmaceutical industries will influence the production of selected pin-pointed molecules, such as β-carotene, peptides, and vitamins. While this is achievable through genetic modification, the ability to increase the yield of one product may inhibit the production of others. This leads back to the original single niche market problem. A more appropriate method would be to optimise one phase of the growth cycle, which would increase the yield of all high-value compounds produced during the selected phase. Understanding the biosynthetic pathways responsible for the production of such compounds is vital to induce optimising stress conditions. An alternative solution would be to use thermal processes such as gasification and pyrolysis to extract high-value compounds from the vapour, liquid, or solid phase during the production of biofuels, in a similar manner to traditional oil refineries.
It is apparent from the literature that a no “one-size fits all” approach can be taken when cultivating microalgae. The biochemical composition is unique to each species and strain within the microalgae taxonomy. Therefore, there appears to be conflicting information regarding microalgae’s ability to establish itself within different markets. This can easily observed in the lipid production between Botryococcus braunii and Chlorella vulgaris.

Reference to Table 2, the total lipid production is 44.97% and 28.07% dry weight respectively. This difference can be explained through their own unique lipid production pathways. B. braunii utilises two biosynthetic pools, one for internal FA, and one for external FA production, whereas C. vulgaris utilises only one biosynthetic pool. Matters become more complicated when other compounds are included, hence the idea of a single species mass producing several high-value compounds is highly unlikely. A possible solution to this problem is through the transfection process, whereby genetic material designed to promote over-production of selected products can be inserted directly into the cells, overcoming the electrostatic barriers of the cell membrane.

The doubling time is a major factor that influences the suitability for large-scale cultivation. Species such as Chlorella vulgaris and Scenedesmus obliquus have short doubling times of 8 – 9 hours, making them suitable candidates for industrial cultivation. Botryococcus braunii however, has a doubling time of 1.4 days, making it one of the slowest growing microalgae recorded. The trade-off, however, is the quantity of high-value compounds available for extraction. While B. braunii lacks the ability to rapidly reproduce compared to C. vulgaris and S. obliquus, it does however, produce significantly higher quantities of lipids than most other species, and similar quantities of other high-value compounds. This has made B. braunii one of the most researched and documented species, alongside Chlorella, Scenedesmus, and Haematococcus.
Traditionally, microalgae cultivation was aimed at the energy sector, as a feedstock for electricity generation, and biofuel production. However, research has shown that the applications for microalgae are more suited towards the pharmaceutical, nutraceutical and medical industries. Revolutionary cancer treatment such as immunotherapy, uses microalgae’s secondary metabolites to activate cytotoxic T cells, that target and destroy cancerous cells. The naturally occurring secondary metabolites such as peptides and phenolics, reduce the purification and refinement process for drug manufacturing.

Microalgae will dominate the pharmaceutical and medical industries, as giving an ageing population and increased life-expectancy, the demand for medicine, cures, and vaccinations continues to grow. *Chlorella* is at the forefront of biomedical and pharmaceutical research, producing substantial quantities of peptides, polyphenols, and carotenoids. Currently these high-value compounds are being used in preventative medicine for cardiovascular disease, degenerative neuropathies, anti-inflammatory disease, and cancer treatment. The antioxidant properties of these high-value compounds are of particular interest in cancer prevention treatment since the formation of cancerous cells begins with a free radical initiation reaction, the presence of peptides and polyphenols within the cardiovascular system provide the required electrons to terminate the formation of cancer cells.

Microalgae present a sustainable and environmentally friendlier alternative to several commodities derived from both plants and animals. However, there has been a limited number of selected strains used in current research, namely: *Chlorella*, *Botryococcus*, *Haematococcus*, and *Dunaliella*. A shift from a single niche to a widespread market would require extensive research and development by both academics and engineers, with a focus on alleviating large-scale production bottlenecks. This can be done by strain genetic engineering, whereby enhanced production of targeted metabolites, such as peptides and polyphenols would increase the economic performance of microalgae cultivation and processing. Advancements in genetic
manipulation should be based on understanding the biosynthesis pathways responsible for metabolite production, to reduce the inhibition of one product against another.

The biorefinery concept is crucial in developing sustainable and competitive microalgae derived products. Several concerns need to be addressed to mitigate the loss of product, reduce energy consumption, and increase metabolite production. While many studies discuss the optimisation of cultivation conditions, bioactive compound applications, and metabolite profiling, few have considered the long-term impacts of maintaining cultures and preventing product degradation. These studies are important to identify areas of high product loss, high energy consumption, and ways to improve process designs in the future.

Furthermore, the development of a mathematical based-model to improve the scalability of lab-scale research to industrial-scale processes would be advantageous in performing viability studies that would assess the economic and environmental sustainability of any proposed design. While a computational model would provide a potential vetting system, the development of such a model would require extensive data to determine the interactions of several input variables, this can be done through full factorial design simulations using design of experiment methodologies. The bottleneck lies with the no “one-size fits all” approach to different species of microalgae. Hence computational models would be species specific and would require extensive research and development, that currently isn’t available yet.

6 – Conclusion

Freshwater eukaryotic microalgae produce a wide range of high-value compounds, with applications in the energy, nutraceutical, pharmaceutical, food and medical industries. The diversity of microalgae makes it an attractive environmentally friendly alternative to synthetic and oil derived products. Traditionally, bioenergy was the main product of interest, however,
public perception of health and wellbeing has shifted the focus to extract secondary metabolites, pigments, and vitamins, that can be used within the pharmaceutical and nutraceutical industries. Microalgae’s ability to adapt to stress conditions secures its theoretical ability to be a main supplier of such compounds. Chlorella, Botryococcus, and Dunaliella are at the forefront of research, and will continue to push the boundary of microalgae cultivation technology to overcome the bottlenecks within the biorefinery concept. Future success of microalgae cultivation will be dependent on a combination of optimized operating conditions, genetic modification, scalability improvements, and biochemical interactions.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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