CHAPTER 1

INTRODUCTION

1.1 MICROALGAE

Microalgae are microscopic algae, typically found in freshwater and marine systems. Microalgae, which are capable of performing photosynthesis, are important for life on earth. They produce approximately half of the atmospheric oxygen and use the greenhouse gas carbon dioxide simultaneously to grow photoautotrophically. They utilize the light energy and carbon dioxide, with a higher photosynthetic efficiency than plants for the production of biomass (Miao & Wu 2006).

The biodiversity of microalgae is enormous and they represent an almost untapped resource. Modern microalgal culture techniques owe their origins to pioneering nineteenth century microbiologists, who first developed methods for the isolation and axenic culture of single phytoplankton species using inorganic salt solutions, leading to initial attempts to rear marine animals on algae-based food chains as long ago as the early 1900s (Anderson 2005, Huntley & Redalje 2007). Mass cultivation of microalgae dates back to the mid-twentieth century, all of the methodologies currently in use can be identified, including open ponds, shallow raceways and enclosed photobioreactors (PBRs). Several taxa of microalgae are also mass cultured for the production of specific extracts, such as β-carotene from Dunaliella salina.
Microalgae have important applications because of their nutritional and dietetics properties due to their pigments (carotenes and phycobiliproteins), polyunsaturated fatty acids (eicosapentaenoic and docosahexaenoic acids) (Molina grima et al 1996) and other products of pharmaceutical interest. Some microalgae are also used to remove heavy metals from marine waters and industrial effluents (Schmitt et al 2001). Recent developments in bioprocess engineering and in-depth understanding of algal physiology have paved the way for current initiatives to mass culture microalgae for bioenergy and food applications.

Most of the valuable compounds produced by microalgae are secondary metabolites. These metabolites are nothing but organic compounds that do not accumulate or participate directly in growth or development, but appear under stress conditions (Croteau et al 2000). Secondary metabolite production is widespread and their multigenic biosynthetic pathways are preserved in nature, indicating that secondary metabolites assist in the survival of the organisms that produce those (Demain & Fang 2000). Nevertheless, many of their specific functions remain unknown.

1.1.1 Microalgae – Isochrysis galbana

Figure 1.1 Image of the microalgae species, Isochrysis galbana
Scientific classification

Domain: Eukaryota  
Kingdom: Chromalveolata  
Phylum: Haptophyta  
Class: Pyrmnesiophyceae  
Order: Isochrysidales  
Family: Isochrysidaceae  
Genus: Isochrysis  
Species: I. galbana

The golden-brown flagellate *I. galbana* (Figure 1.1) is an important marine microalga, as well as it has favourable lipid composition (rich in ω-3 PUFA). This Haptophyta is of substantial interest in aquaculture, principally to feed mollusk larvae, as well as fish and crustaceans in the early stages of growth (Pernet et al 2003). Other important biomolecules such as sterols, tocopherols, pigments and pharmaceuticals, are also synthesized by this microalga (Fabregas & Herrero 1990).

Morphological features

- Golden brown
- Cells spherical with two flagella
- 5-6 µm in length; 2-4 µm wide
1.1.2 Microalgae — *Nannochloropsis oculata*

![Image of microalgae species](image)

**Figure 1.2 Image of the microalgae species, *Nannochloropsis oculata***

**Scientific classification**

- **Domain**: Eukaryota
- **Kingdom**: Chromalveolata
- **Phylum**: Heterokontophyta
- **Class**: Eustigmatophyceae
- **Order**: Eustigmatales
- **Family**: Eustigmataceae
- **Genus**: Nannochloropsis
- **Species**: *N. oculata*

*Nannochloropsis oculata* (Figure 1.2) is known for its oleaginous characteristics, with a specific high content of eicosapentaenoic acid (EPA), and has been investigated for applications in aquaculture as larval feed and as feedstock for biodiesel (Pal et al. 2011).

**Morphological features**

- Green colored
Cells are spherical in shape
Measures approximately 2-4 µm in diameter

1.2 MICROALGAE GROWTH REQUIREMENTS

Several strategies have been applied to improve the microalgae growth and lipid content. The growth rate is mainly dependent on algal species, light intensity and temperature. Cell division slows down when nutrients, light, pH, CO₂ or other physical and chemical factors becomes limiting. In order to achieve an efficient production with maximum growth rates, the optimum culture conditions must be understood and controlled.

1.2.1 Light Requirements

Microalgae are photosynthetic organisms. They assimilate inorganic carbon and transform it to organic matter. Light is the energy source that drives this reaction. It is important to identify the right type of light source with appropriate wavelengths in order to achieve the high level of photosynthetic efficiency. The light requirement for algae is depends upon the major pigments present in the algal cell. Chlorophyll a, b and c absorb/harvest specific regions in the photosynthetic active region (PAR) of visible light.

1.2.2 CO₂ Addition and pH Control

The addition of CO₂ must not change the pH of the culture drastically. The algal cells will assimilate the dissolved CO₂ and transform it into organic matter. However, if the growth rate is less and cells do not remove the CO₂ at the expected rate, the increasing level of dissolved CO₂ will eventually decrease the pH in the culture medium. The pH range for most of the cultured species varies between 7 and 9 (Lavens & Sorgeloos 1996).
1.2.3 O$_2$ Removal

High Oxygen concentration around algal cells is undesirable. High oxygen concentration results in photooxidative damage to algal cells, hence the oxygen concentration should be maintained below 40 % of air saturation value (Chisti 2007).

1.2.4 Mixing

The intensity of mixing strongly contributes to algal growth in two primary ways;

i) improvement of productivity by increasing the frequency of cell exposure to light and dark volumes of the reactor

ii) increasing the mass transfer rate between the nutrients and cells

However, the mixing parameter has to be optimized precisely because high mixing rate will result in cell death from shear stress.

1.2.5 Nutrient Requirements

Microalgae cultures must be enriched with adequate nutrients in order to sustain growth. Macronutrients include nitrate, phosphate and silicate. Silicate is mainly used by diatoms, which utilize this compound for production of an external cell covering. Micronutrients include various trace metals (Zn, Co, Cu, Mo, Mn, Fe) and vitamins (thiamine, cyanocobalamin and biotin). Two enrichment media namely walne medium (Laing 1991) and f/2 medium (Guillard 1975) are commonly used for the growth of algae in aquaculture.
1.2.6 Temperature Control

Optimum temperature generally varies between 18 and 24 °C for most microalgae (temperate and sub-tropical species) used. Many of the cultured species tolerate temperatures between 16-27 °C. Temperatures lower than 16 °C will most likely result in slow growth, while temperatures above 35 °C will lead to culture collapse.

1.2.7 Salinity Control

In general Marine microalgae are tolerant to changes in salinity. In culture, most species grow best at a salinity that is a bit lower than found in their native habitat (diatoms at 20-25 % and flagellates at 28-30 %).

1.3 CULTIVATION OF MICROALGAE

Open raceway pond system and closed photobioreactor system are the two most commonly used techniques to cultivate microalgae.

1.3.1 Open Ponds

Open pond systems are recognized for being simple, easy to operate and inexpensive (low capital and operating costs). They are easy to clean up after cultivation (Ugwu et al 2008) and low process control is needed. They are good systems for lower value products with large markets. Raceway ponds (Figure 1.3 a), shallow big ponds, circular ponds tanks and closed ponds are currently used in industrial research.

Open ponds are limited by lack of control of key growth parameters including light intensity, temperature, pH and dissolved oxygen concentration. Contamination by predators is another issue involved with open ponds. Several studies have assessed the possibility of microalgae cultivation using the open ponds system. Lee (2001) reported that only a
limited range of microalgae species resistant to be cultured in open ponds due to severe culture environment (Dunaliella-high salinity, Spirulina-high alkalinity, and Chlorella-high nutrition). *Dunaliella salina* is one of the most successful species that have been used for production of carotenoids. Hase et al (2000) achieved a stable photosynthetic efficiency of *Chlorella* sp. and *Chlorophyta* sp. in raceway system. Moreover, Blanco et al (2007) used *Muriellopsis* sp. to produce lutein rich cells in the open tank agitated with a paddle wheel.

The commercial production of *Spirulina* biomass is based almost exclusively on open reactors, especially open raceway ponds (Richmond 1990). Although some problems in production plants are frequently observed, the open cultivation of *Spirulina* is easily accomplished. The tanks are relatively simple to build and to operate, and can have scale size up to 5000 m$^2$ or even more (Tredici et al. 1993).

### 1.3.2 Closed Cultivation System

Closed systems offer advantages of controlling and optimizing microalgal growth more efficiently than in open systems. This can result in higher biomass yield and density.

Product standardization is possible because every element of the production can be controlled by CO$_2$ supply, water supply, temperature, light exposure, culture density, pH and mixing regime. Closed systems offers good control of CO$_2$ transfer and helps minimal CO$_2$ and culture medium loss. Generally photobioreactors are used for cultivation in closed systems.

#### 1.3.2.1 Photobioreactors

Photobioreactor gives a better control on most of parameters compared to open pond systems (Chisti 2007). The controlled environment of
photobioreactor allows a higher productivity to be achieved. Tubular and plate type photobioreactors are commonly used. In tubular photobioreactors, tubing can be arranged in various configurations such as straight line and coiled tubing. The application of tubular photobioreactors in culturing microalgae are external loop (Fig 1.3 b), vertical, horizontal (Fig 1.3 c) and helical which are reported in number of studies (Richmond et al 1993, Hall et al 2003). However, photobioreactors have a high initial cost and are very specific to the physiology of microalgae strain being cultivated (Harun et al 2010).

Closed photobioreactors have been used in the cultivation of _Porphyridium, Phaeodactylum, Nannochloropsis, Chlorella, Haematococcus_ and _Tetraselmis_, among others (Chini-Zittelli et al. 1999; García-Malea et al. 2009; Rebollos Fuentes et al. 1999; Acien et al. 2001; Ugwu et al. 2002).
Figure 1.3 Reactor configurations for microalgae cultivation a) raceway pond, b) external loop tubular reactor, c) horizontal tubular reactor (Harun et al 2010)

1.4 MODES OF CULTIVATION

Traditional microalgae cultivation techniques, which rely on photosynthetic microalgae are grown in outdoor ponds or indoors under artificial lights. They are generally expensive due to the relatively low biomass concentrations achieved, large operating costs, often limited by insufficiency of light caused by mutual shading of cells and culture
inefficiencies. Several reports (Wen & Chen 2003, Ceron Garcia et al 2006, Bumbak et al 2011, Choi et al 2011) have outlined that mixotrophic and heterotrophically grown microalgal biomass is potentially a cost-effective alternative. To enhance essential fatty acid production by microalgal culture, the development of mixotrophic and heterotrophic growth process is desirable.

1.4.1 Heterotrophic Cultivation

Heterotrophic cultivation of microalgae using organic carbon source offer several advantages over photoautotrophic cultivation including elimination of light, good control of cultivation process, high biomass and lipid content in cells (Miao & Wu 2006). Bioreactor operation and maintenance is relatively simple and can be performed under strict axenic conditions. Cell masses obtained under heterotrophic conditions are higher because the energy density of the carbon source is higher in comparison with carbon dioxide.

However, heterotrophic cultures also have drawbacks: there is a limited number of microalgal species that can grow heterotrophically; energy expenses and costs by adding an organic substrate are higher; growth can be inhibited by an excess of organic substrate; and light-induced metabolites cannot be produced.

A microalga suitable for heterotrophic culture should have the following physiological abilities such as 1) divide and metabolize without light, 2) grow on easily sterilized culture media, 3) adapt rapidly to environmental changes and 4) withstand the hydrodynamic stresses generated in stirred tank bioreactors and peripheral equipment (Azma et al 2011). Several strains of algae, including Chlorella protothecoides, Galdieria sulphuraria, Nitzschia laevis and Cryptecodinium cohnii have been studied under heterotrophic growth conditions to achieve higher quantities of dry

Heterotrophic cultivation of Neochloris oleoabundans using glucose as a carbon source was carried out by Sanchez et al (2013). Maximum cell biomass of 9.2 g/L and lipid productivity of 528.5 mg/L/day was achieved using batch cultures at a C/N ratio of 278.

The effectiveness of various physical and chemical methods for the removal of contaminants from the microalgae, Tetraselmis suecica culture was investigated by Azma et al (2010). The adaptation of growth from photoautotrophic to heterotrophic condition was achieved by repeated photoautotrophic cultivation with the sequential reduction in illumination time. Finally the culture was inoculated into the medium containing 10 g/L glucose, incubated in total darkness to obtain heterotrophic cells. In another study carried out by Azma et al (2011), optimization of medium composition for the improvement of heterotrophic cultivation of green microalgae, Tetraselmis suecica, was performed using response surface methodology (RSM). In this study, heterotrophic cultivation of T. suecica was conducted in total darkness using walne medium formulated with natural sea water. Initially, the effect of two types of carbon sources (glucose and sodium acetate) and various types of nitrogen sources (peptone, yeast extract, meat extract, malt extract, urea, sodium nitrate and ammonium nitrate) on growth of T. suecica was studied. Then the concentration of medium component that was found to significantly influence the heterotrophic growth of T. suecica (glucose, peptone, yeast extract and meat extract) was further optimized using RSM. The medium that consists of 5.78 g/L glucose, 9 g/L peptone, 4.48 g/L yeast extract and 3.01 g/L meat extract was found optimal for heterotrophic cultivation of T. suecica. The final cell concentration (28.88 g/L) obtained in heterotrophic cultivation using this optimized medium was about 3 to 2 times
higher than obtained in photoautotrophic culture (8.40 g/L) and non-optimized medium for heterotrophic cultivation (13.81 g/L), respectively. In addition, the cell yield based on glucose consumption (9.31 g cell/g glucose) was increased by about 3 times as compared to non-optimized medium (3.61 g cell/g glucose).

Heterotrophic growth of microalgae *Chlorella* sp. KKU-S2 for lipid production has been investigated using molasses as carbon substrate and yeast extract as nitrogen source by Leesing (2011). Culture medium supplemented with 30 g/L molasses and 2.0 g/L yeast extract resulted in high both 1.796 g/L/d volumetric cell mass production rate and 0.522 l/d specific growth rate.

### 1.4.2 Mixotrophic Cultivation

Mixotrophic growth culturing was performed using photosynthesis as the main energy source, though both organic compounds and CO$_2$ were essential (Berninger et al 1992). Mixotrophic mode is considered better and more sustainable than the heterotrophic mode as it utilizes solar energy for lipid production and helps in biosequestration of CO$_2$ (Miao & Wu 2006).

Under mixotrophic conditions some microalgae have higher growth rate than photoautotrophic conditions (Cheirsilp & Torpee 2012) and produce compounds that are synthesized during both heterotrophic and phototrophic conditions. It has been considered as a potential alternative strategy for producing wide range of economically viable microalgal products in a short span of time. Mixotrophic cultivation can be used as an efficient method for seed cells production, which can be used as inoculums in the subsequent phototrophic open pond cultivation for algal biomass and lipid production.

*Nannochloropsis* sp. was grown under mixotrophic conditions with glucose as carbon source under fed batch conditions by Xu et al (2004). The
biomass weighed 1.1 g dry wt/L after 10 days culture, which was 40 % higher than the batch culture (0.8 g dry wt/L). The maximum biomass (1.2 g dry wt/L) was obtained with the supplement of the mixture of glucose and nitrate solution. The EPA yields of Nannochloropsis sp. after 10 days growth in the fed-batch cultures were 52, 43 and 56 mg/L with addition of nitrate, glucose and both together, respectively.

Mixotrophic study of Phaeodactylum tricornutum UTEX 640 was carried out by Garci et al (2000) in 1 L batch cultures by supplementing glycerol as carbon source. The optimal glycerol concentration was 0.1 M. The maximum biomass concentration of 16.2 g/L and maximum biomass productivity of 61.5 mg/L/h were achieved. These values were 9 and 8-fold higher than in the photoautotrophically grown control.

Mixotrophic cultivation of marine Chlorella sp. was performed by choi et al (2011) using methanol as carbon source, which may also be helpful in maintaining the sterility of the medium. Methanol concentration exceeding 1 % was found to be toxic. The optimal methanol concentration was determined to be 1 % (v/v) for both cell growth and lipid production when supplying 5 % CO$_2$ with 450 μE/m$^2$/sec of continuous illumination. The maximal cell biomass and total lipid production were 4.2 g dry wt/L and 17.5 % (w/w), respectively, compared to 2.2 g dry wt/L and 12.5 % (w/w) from autotrophic growth.

In order to reduce microalgal production costs, it is imperative to find the cheap organic substrates that meet the nutritional requirements of oleaginous algae. Few studies have used cheap substrates such as cassava starch hydrolysate (Wei et al 2009) and corn powder hydrolysate (Xu et al 2006) as glucose substitutes resulting in higher lipid yields.

Mixotrophic cultivation of Chlorella vulgaris using hydrolyzed cheese whey solution was carried out by Abreu et al (2012). Mixotrophic microalgae
showed higher specific growth rate, final biomass concentration and productivities of lipids, starch and proteins than microalgae cultivated under photoautotrophic conditions. Moreover, supplementation of the inorganic culture medium with hydrolyzed cheese whey powder solution led to a significant improvement in microalgal biomass production and carbohydrate utilization when compared with the culture enriched with a mixture of pure glucose and galactose, due to the presence of growth promoting nutrients in cheese whey.

1.5 MICROALGAE OPTIMIZATION STUDIES

1.5.1 Response Surface Methodology

To develop the process technology in microalgal cultivation, single-factor experiment was applied to optimize the cultivation conditions of microalgae. However, the traditional method is time-consuming and has a poor performance for optimizing a large number of parameters. Besides, it might lead to erroneous conclusions, since interactions between factors are missed (Abdel-Fattah et al 2005). For this reason, statistical methods have been developed in microalgal system. Among these, response surface methodology (RSM) coupled with central composite design (CCD), is a comprehensive and multi-functional tool for designing experiments, building models, and analyzing the effects of multiple factors and their interactions (Chen et al 2012).

The optimal conditions for the effective mixotrophic culture of *H. pluvialis* were partially established using response surface methodology (RSM) by Jeon et al (2006). The light intensity and acetate concentration were selected as major factors affecting the algal growth rate. The first factorial design was performed with the light levels of 80–120 µ/m²s and 40–70 mM acetate. As a result, the optimum conditions were not present within the domain of the first experimental design. An ascent line plotted from the
first design showed that the optimum conditions existed within a region of lower concentration of acetate, but similar intensity of light. On the basis of the ascent line, additional experiments were carried out using a central composite design, which enhanced the growth rate up to 0.243 g/L/day at a light intensity of 170 µ/m²s and acetate concentration of 30 mM.

Optimization of medium composition for heterotrophic cultivation of green microalgae, *Tetraselmis suecica*, was performed using RSM by Azma et al (2010). The medium that consists of 5.78 g/L glucose, 9 g/L peptone, 4.48 g/L yeast extract and 3.01 g/L meat extract was found optimal for heterotrophic cultivation of *T. suecica*.

Xie et al (2012) carried out RSM with CCD for the optimization of heterotrophic cultivation of *Chlorella* sp. The optimum conditions of glucose concentration, sodium nitrate concentration and temperature were 26.2 g/L, 2.06 g/L and 28.18 °C, respectively.

Optimization of biomass and arachidonic acid (ARA) in *Aureispira maritima* was carried out by saelao et al (2011). Four important parameters (Tryptone, temperature, pH & agitation rate) were initially screened using plackett-burman design and were subsequently optimized using RSM. Maximum of 2.05 g/L biomass production was achieved with 21.50 mg/g ARA yield.

1.6 MICROALGAE IN AQUATIC FOOD CHAIN

Microalgae are by far the most abundant primary producers that can be found in most aquatic systems. They photosynthetically convert light energy and carbon dioxide (CO₂) into biomass such as carbohydrates (Park et al 2011), proteins (Becker et al 2007) and lipids (Harwood et al 2009). They are widely used as food for many species of larval and juvenile fish, shellfish and shrimps, either as live or dry feed. Several studies have shown that they are
rich sources of marine protein and lipids. Importantly, microalgae are also the primary producers of EPA and DHA that are eventually accumulated through the various trophic levels. Changes in microalgal lipid content are carried on up the food chain (Figure 1.4), impacting the growth and dietary make-up of zooplankton, crustacean larvae, mollusc and some fish (Brown et al 1997). This subsequently affects the accumulation of EPA and DHA fatty acids in higher organisms and humans. Consequently, lipid profiles in microalgae play a vital role in maintaining the integrity of the world’s aquatic food webs.

Figure 1.4 Role of microalgae in marine and freshwater food chain (Adarme-Vega et al 2012)
1.6.1 The Nutritional Importance of Microalgae in Aquaculture

Microalgae are essential to the aquaculture industry which has grown substantially over the last 10 years (Foster et al 2008). The successful cultivation of oysters, scallops and mussels is dependent on the ω-3 fatty acids from microalgal feedstock. The polyunsaturated omega-3 fatty acids EPA and DHA derived from microalgae (e.g. Isochrysis, Tetraselmis, Chaetoceros, Thalassiosira, Nannochloropsis) are also known to be essential for healthy development of various bivalve larvae (Caers et al 2003, Soudant et al 1996). Prior research on the scallop Pecten maximus has shown a direct relationship between the fatty acid profile of female gonads and the fatty acid composition of the eggs (Utting et al 1998). The increase of EPA and DHA from an algal diet significantly increased the concentration of fatty acids in the digestive gland (78 %) of scallops as well as the female (57 %) and male gonads (51 %). It appears that dietary lipids are stored in the digestive gland and are later transferred to the developing female gonad. These dietary lipids are then incorporated into the eggs and can significantly improve their quality. This in turn improves the hatching rate of eggs and hatching rates have been linked to high contents of EPA and DHA (Soudant et al 1996). Aside from bivalve culture, microalgae are also used as food additives to improve the flesh color of salmon (Torrissen et al 1986), as well as inducing a range of other biological activities such as survival and resistance (Schenk et al 2008). At present, the most widely cultured species for aquaculture hatcheries and nurseries include Chaetoceros calcitrans, Isochrysis galbana, Pavlova lutheri, Pseudoisochrysis paradoxa, Tetraselmis suecica and Skeletonema costatum. Other genera include Spirulina, Dunaliella, Chlorella, Thalassiosira, Isochrysis and Nannochloropsis (Brown et al 2002).
1.7 CAPTURING CO₂ WITH MICROALGAE

Over the past several decades, global warming has become a serious threat to both humans and nature (Brennan & Owende 2010). The rise in global temperature is attributed to the high amount of carbon dioxide (CO₂) gases in the atmosphere (Florides & Christodoulides 2009). CO₂ is emitted from the burning of fossil fuels for electricity, transport and industrial processes (Brennan & Owende 2010). Due to the serious threat of global warming, the Kyoto Protocol in 1997 proposed a reduction of greenhouse gases by 5.2 % based on the emissions in 1990. Since then, many CO₂ mitigation options have been considered to meet the proposed target. The various strategies suggested can be classified into two main categories: chemical reaction-based approaches and biological mitigation. Chemical reaction-based strategy captures CO₂ by reaction with other chemical compounds before the CO₂ is released to the atmosphere. The disadvantage of this method is that the chemical reactions are very energy-intensive and costly. Furthermore, both CO₂ and the wasted chemical compounds need to be disposed of. On the other hand, biological mitigation is deemed to be more favourable as it not only captures CO₂ but also generates energy through photosynthesis (Wang et al 2008). This method will be much simpler than physical CO₂ sequestration (Schenk et al 2008). Photosynthesis is carried out by all plants and any photosynthetic microorganisms. Even though the use of plants to capture CO₂ is viable, it is by no means efficient owing to its slow growth rate. On the other hand, microalgae as photosynthetic microorganisms are able to capture solar energy and CO₂ with an efficiency of 10 to 50 times greater than that of higher plants (Wang et al 2008). Microalgae include both prokaryotic cyanobacteria and eukaryotic unicellular algae (Brennan & Owende 2010). The structural and functional simplicity of these microorganisms makes them a better choice for research purposes than any other terrestrial plants (Becker 1994). In addition, microalgae have rapid
growth rates and higher productivities than any other plant systems. Microalgae can also grow in variable environmental conditions (Wang et al 2008). Apart from CO$_2$ and sunlight, microalgae also need nutrients, trace metals and water to grow (Packer 2009). In short, biomass from microalgae cultivation is produced based on the following reaction:

$$\text{CO}_2 + \text{H}_2\text{O} + \text{nutrients} + \text{light energy} \rightarrow \text{Biomass} + \text{O}_2$$

Unlike plants, microalgae can be cultivated with waste or brackish water despite its abundance of heavy metals and pathogens. The nitrogen and phosphorous in waste water can be directly used by the microalgae as nutrients. Hence, use of chemicals and freshwater needed for algal cultivation can be reduced. It also means that it is entirely feasible to incorporate wastewater treatment process with microalgal cultivation (Brennan & Owende 2010, Wang et al 2008). Microalgae can capture CO$_2$ from the atmosphere, from industrial gases (i.e., power plant flue gases) and in the form of soluble carbonates (i.e., Na$_2$CO$_3$ and NaHCO$_3$) (Wang et al 2008, Benemann 1997). Efficiency of CO$_2$ capture by microalgae increases with power plant flue gases that have up to 15 % of CO$_2$ (Wang et al 2008) and studies have shown that microalgal species (such as *Scenedesmus sp.* and *Chlorella sp.* can tolerate 10 % to 30 % CO$_2$ in its gas supply (Hanagata et al 1992). Therefore microalgal cultivation can be synergistically located adjacent to a power plant where the CO$_2$ emitted from such a plant can be conveniently sequestered by the algae as a source of their nutrients (Brennan & Owende 2010).

### 1.8 PRODUCTS FROM MICROALGAE

#### 1.8.1 Pigments

The most commercially valuable groups of pigments are carotenoids and phycobilins with several available products derived mainly from green
microalgae (e.g. Dunaliella) and Cyanobacteria (e.g. Spirulina) (Prasanna et al 2007).

Carotenoids are divided into carotenes and xanthophylls. There are over 400 known carotenoids, but only a few are used commercially, the two main compounds being \( \beta \)-carotene and astaxanthin. Chiefly carotenoids are as food colourants and supplements for human and animal feeds (Becker 1994, Spolaore et al 2006).

The average concentration of carotenoids in most algae is only 0.1–2%, but Dunaliella when grown under the right conditions of high salinity and light intensity will produce up to 14% \( \beta \)-carotene (Becker 1994, Singh et al 2005, Borowitzka 2006, Spolaore et al 2006). Dunaliella is, therefore, well suited to the commercial production of \( \beta \)-carotene and several industrial production plants are in operation around the world including Australia, Israel, USA and China (Spolaore et al 2006). The major producer is Cognis Nutrition and Health, whose farms cover 800 ha in Western Australia and produce \( \beta \)-carotene extracts together with algal powder for human and animal use. The prices of these products in 2004 varied from US$300 to US$3000/kg (Spolaore et al 2006).

Astaxanthin is another carotenoid that can be derived from algae and is principally used in fish farming and as a dietary supplement or anti-oxidant. Astaxanthin can be produced by Haematococcus, a freshwater alga that normally grows in puddles, birdbaths and other shallow fresh water depressions. Haematococcus can contain up to 3% astaxanthin, but it requires a two stage culture process which is not suited to open pond cultivation. The first stage of the process is designed to optimize algal biomass (green-thin walled flagellated stage with optimum growth at a temperature 22–25 °C) and the second stage (thick walled resting stage) under intense light and nutrient poor conditions during which astaxanthin is produced (Borowitzka 2006, Spolaore et al 2006). Commercial production is being carried out in Hawaii,
India and Israel, where Algatech sell a crushed Haematococcus biomass on the pharmaceutical market (Borowitzka 2006, Spolaore et al 2006).

Phycobiliproteins, are deeply coloured (red or blue), water soluble, complex, proteinaceous compounds. Phycobiliproteins (phycoerythrin, different phycocyanins and allophycocyanins) from red algae and cyanobacteria have a long tradition of use as dyes in food, cosmetics and as fluorescent markers in biomedical research. Phycobiliproteins can be commercially produced from Spirulina and the red microalgae Porphyridium and Rhodella (Becker 1994, Singh et al 2005, Borowitzka 2006, Spolaore et al 2006). Dainippon Ink and Chemicals produces a blue food colourant from Spirulina, called Lina blue, that is used in chewing gum, ice slush, sweets, soft drinks, dairy products and wasabi (Spolaore et al 2006, Raja et al 2008).

1.8.2 Biodiesel

Microalgae are potential to be used as a raw material for biodiesel production because algae are the most efficient biological producer of oil on the planet and a versatile biomass source and may soon be one of the Earth’s most important renewable fuel crops. This is mainly due to the higher photosynthetic efficiency, higher biomass productivities, a faster growth rate than higher plants (which is also important in the screening step), highest CO₂ fixation and O₂ production, growing in liquid medium which can be handled easily. It can be grown in variable climates and non-arable land including marginal areas unsuitable for agricultural purposes (e.g. desert and seashore lands), in non-potable water or even as a waste treatment purpose, use far less water than traditional crops and do not displace food crop cultures. Their production is not seasonal and can be harvested daily (Chisti 2007). As a matter of fact, average biodiesel production yield from microalgae can be 10 to 20 times higher than the yield obtained from oleaginous seeds and/or vegetable oils (Chisti 2008). Microalgal lipids are mostly neutral lipids with
lower degree of unsaturation. This makes microalgal lipids a potential replacement for fossil fuel.

### 1.8.3 Bioethanol

Microalgae biomass can potentially be utilized for bioethanol production as it contains carbohydrates and proteins that can be used as carbon sources for fermentation. They are fermented in anaerobic and dark environment to produce ethanol. The ethanol produced from fermentation can be purified to be used as fuel and produced CO$_2$ was recycled to algae cultivation ponds as a nutrient to grow microalgae.

### 1.8.4 Omega-3 Fatty Acids

Algal fatty acids are either saturated (SFAs), monounsaturated (MUFAs), or polyunsaturated acids (PUFAs); the latter refer to chains longer than 18 carbon atoms which are essential to human and animal nutrition and which must be ingested from food sources. PUFAs include n–6 (ω-6) and n–3 (ω-3) fatty acids, for example arachidonic (AA) and eicosapentaenoic (EPA) acid, respectively. Commercially produced microalgal PUFAs with reported bioactivities of particular interest are EPA (Figure 1.5) (anti-inflammatory, rheumatoid arthritis, and treatment of heart disease), DHA and palmitoleic acid (reduce risk of certain heart diseases), oleic acid (antioxidant capacity), and linolenic acid and palmitic acid with reported anti-microbial activity (Plaza et al 2009).
It appears that lipid class and Fatty acid (FA) composition of microalgae are highly variable during culturing. Dunstan et al (1993) investigated the changes in lipid class with FA composition of *P. lutheri* and *Isochrysis sp.* grown in 100 litre bags, either as logarithmic and stationary phase batch cultures or as semi-continuous cultures, and found that they changed depending culture technique and growth phase. Pernet et al (2003) found high variability in both lipid class and FA composition of *C. muelleri* and *Isochrysis sp.* in a semi-continuous system. In a continuous bag culture system some variability in FA composition were observed between replicate bags of *Isochrysis sp.*, *P. lutheri* and *C. muelleri*, but the production of PUFA increased over time (Jacobsen et al 2010).

1.8.5 Stable Isotope Biochemicals

Microalgae are well suited as a source of isotopically labelled compounds due to their ability to incorporate stable isotopes from relatively inexpensive inorganic molecules into high value isotopic organic chemicals (Raja et al 2008). The market for these chemicals is in excess of US$ 13 million (Spolaore et al 2006). Spectra Stable Isotopes, now part of Cambridge Isotope Laboratories, sells marked amino acids at up to US$ 5900/g and marked nucleic acids at US$ 28/ mg (Spolaore et al 2006).

**Figure 1.5 Structures of EPA & DHA (Plaza et al 2009)**

![EPA (20:5 ω-3)](image)

![DHA (22:6 ω-3)](image)
1.8.6 Animal Feed

Microalgae are an important food source and feed additive in the commercial rearing of many aquatic animals (Borowitzka 2006). Over 30% of the current world algal production is sold for animal feed and over 50% of the world production of Spirulina as feed supplements. Many studies have shown the suitability of algae as a potential animal feed and as a replacement for conventional protein sources such as soybean and fish meal (Becker 1994, Spolaore et al. 2006).

1.9 Downstream Processing Techniques

The common methods that have been employed to extract the lipids from microalgae include oil press, homogenization, milling, solvent extraction, supercritical fluid extraction and ultrasonic assisted extraction.

The effectiveness of harvesting and extraction techniques depends on the microalgal strain's physical characteristics (e.g. cell size and cell wall properties) and the use of the end product. In aquaculture, microalgae are used as a fresh product or as dry pellets which preserve the nutritional content of microalgae (Borowitzka 1997, Benemann 1992, Reitan et al. 1997). In this case, microalgal bio-mass is first de-watered either by filtration, dissolved air flotation, flocculation or sedimentation and then dried to form pellets or directly administrated to livestock (Reitan et al. 1997). When produced for the pharmaceutical industry, further extraction and purification processes are required. Currently, methods such as supercritical fluid extraction, winterization and fractional (molecular) distillation are used for the extraction and purification of PUFA from microalgae (Andrich et al. 2005, Herrero et al. 2006).
1.9.1 Mechanical Cell Disruption

Mechanical disruption techniques such as pressing and homogenization essentially involve using high pressures to rupture cell walls, in order to recover the oil from within the cells.

Milling on the other hand, uses grinding media (consisting of small beads) and agitation to disrupt the cells. Biomass agitation within the grinding media (beads) results in damaged cells, where the degree of disruption depends mostly on contact between biomass & beads; size, shape and composition of the beads, and strength of the microalgal cell walls (Doucha & Livansky 2008). Bead milling is generally used in conjunction with solvents to recover oil. This is most effective and economical when cell concentrations are significant and when extracted products are easily separated after disruption. Typically, this type of cell disruption is most effective and energy-wise when biomass concentrations of 100 to 200 g/L are used (Greenwell et al 2010).

1.9.2 Solvent Extraction Method

Organic solvents, such as benzene, cyclohexane, hexane, acetone and chloroform have shown to be effective when used on microalgae paste. They degrade microalgal cell walls and extract the oil because oil has a high solubility in organic solvents (Harun et al 2010). However, it is feasible (at least using Botryococcus braunii) to extract oil from microalgal biomass without damaging cell walls, as long as the solvent is not toxic to the cells (Banerjee et al 2002). The desired solvent for extraction should be insoluble in water, should solubilize the compound of interest, inexpensive, reusable and have a considerable different density than water.
An efficient extraction requires the solvent to fully penetrate the biomass and to match the polarity of the targeted compound(s) (i.e. non-polar solvent such as hexane for extracting non-polar lipids). One way to facilitate this is to mechanically disrupt the cells prior to exposing them to the solvent (Cooney et al 2009). Alternatively, solvent extraction can be enhanced by using organic solvents at different temperatures and pressures above their boiling point. This is called as accelerated solvent extraction (ASE), and it is applicable to solid and semi-solid matrices, thus requiring samples to be dried prior to extraction (Cooney et al 2009). Traditionally, lipids have been extracted from biological matrices using a combination of chloroform, methanol and water (Bligh & Dyer 1959). This procedure is known as the Bligh and Dyer method. This is originally designed to extract lipids from fish tissue and has been used as a benchmark for comparison of solvent extraction methods.

1.9.3 Supercritical Fluid Extraction

An extraction method that has gained acceptance in recent years is the use of supercritical fluids to extract high-value products from microalgae. This is because it produces highly purified extracts that are free of potentially harmful solvent residues, extraction and separation are quick, as well as safe for thermally sensitive products (Sahena et al 2009). Supercritical fluid extraction involves the use of substances that have properties of both liquids and gases (i.e. CO$_2$) when exposed to increased temperatures and pressures. Carbon dioxide is favored because of its relatively low critical temperature (31.1 °C) and pressure (72.9 atm) (Cooney et al 2009). Supercritical CO$_2$ extraction efficiency is affected by four main factors: pressure, temperature, CO$_2$ flow rate and extraction time (Andrich et al 2006). These factors, along with the use of modifiers (most commonly ethanol as a co-solvent), can be altered and adjusted to optimize extractions.
Supercritical CO$_2$ extraction, in combination with a small concentration (usually 10–15%) of ethanol as a co-solvent, has shown to give comparable lipid yields to the benchmark Bligh and Dyer solvent system of chloroform, methanol and water when extracting oil from *Arthrospira maxima*, as well as *Spirulina platensis* (Mendes et al. 2006).

Since CO$_2$ is a gas at room temperature, it is easily removed when extraction is completed, thus it is safe for food applications (Mendes et al. 2006), and it can safely be recycled, which is an environmental benefit (Sahena et al. 2009). One restriction to supercritical CO$_2$ extraction is the level of moisture in the sample. It acts as a barrier against diffusion of CO$_2$ into the sample, and diffusion of lipids out of the cells. Samples should be dried prior to supercritical fluid extraction (Sahena et al. 2009).

1.9.4 Ultrasound Assisted Extraction

Through cavitation, ultrasonic-assisted extractions can recover oils from microalgal cells. Cavitation occurs when vapour bubbles of a liquid form in an area where pressure of the liquid is lower than its vapour pressure. These bubbles grow when pressure is negative and compress under positive pressure, which causes a violent collapse of the bubbles. If bubbles collapse near cell walls, damage can occur and the cell contents are released (Wei et al. 2008). Both ultrasound and microwave-assisted methods improve extractions of microalgae significantly, with higher efficiency, reduced extraction times and increased yields, as well as low to moderate costs and negligible added toxicity.
1.10 OMEGA-3 FATTY ACIDS

1.10.1 Current Sources

Currently, the principal source of EPA and DHA for human consumption is from marine fatty fish such as salmon, mullet and mackerel (Gunstone 1996), whereas plant sources such as canola, walnut, soybean, and flaxseed contain α-linolenic acid (ALA 18:3 ω3). Declining fish stocks, fishy odor, seasonal variations in quality as well as the presence of pollutants such as heavy metals and PCBs in fish (Domingo & Bocio 2007) may lead to health problems during the consumption of fish for the requirement of EPA and DHA. Therefore, other dietary sources of EPA and DHA are being sought. In addition, fish oil is not favourable for vegetarians and the odour makes it unattractive. Variety of alternative EPA and DHA sources such as bacteria, fungi, plants and microalgae are currently being explored for commercial production. Fungi require an organic carbon source and typically long growth periods (Barclay et al 1994), plants need arable land, have longer growth times and have no enzymatic activity for producing long chain PUFAs EPA and DHA, unless genetically modified (Ursin 2003). Fish get the ω-3 fatty acids from their prey organisms (smaller fish, crustaceans, molluscs or fish feed containing fish oil in aquaculture) which in turn are enriched by consuming microalgae. They are the primary natural producers of long chain polyunsaturated fatty acids (LCPUFA) including EPA and DHA. Micro-algae are in the bottom of the food pyramid and very high photosynthetic capacity. In that sense, harvesting ω-3 fatty acids directly from microalgae is most environmentally efficient and sustainable production practice.

Autotrophic and mixotrophic microalgae fix atmospheric carbon dioxide during photosynthesis. It can potentially grow on non-arable land and have short harvesting times (Rubio-Rodríguez et al 2010, Schenk et al 2008).
In particular heterotrophic microalgae are well established as an alternative source of DHA and are added to infant milk formula or other food.

### 1.10.2 Health Benefits

Omega-3 fatty acids (EPA & DHA) represent an important structural component of human cell membranes, particularly neuronal cells (Brunner 2006). EPA & DHA act as precursors in the synthesis of prostaglandins, leukotrienes, tromboxanes and resolvins. They bind to specific protein receptors and signal cellular physiological responses to inflammation, vasodilation, blood pressure, pain and fever, playing an important role in the prevention of cardiovascular diseases, type II diabetes, ocular diseases, arthritis and cystic fibrosis (Simopolous 1999).

With regards to cardiovascular health, regular consumption of omega-3 fatty acids can help reduce the risk of hypertension, thrombosis, myocardial infarction and cardiac arrhythmias (Horrocks & Yeo 1999). This occurs because omega-3 fatty acids increase the high-density lipoprotein/low-density lipoprotein (HDL/LDL) ratio and decrease the total cholesterol/ HDL ratio (Horrocks & Yeo 1999). In addition to cardiovascular benefits, omega-3 fatty acids have also demonstrated positive effects on brain function and the nervous system (Simopoulos et al 2009). In pregnant women, the adequate intake of EPA and DHA is crucial for healthy development of the fetal brain (Damude et al 2008). In infants, arachidonic acid (ARA), an omega-6 fatty acid, and DHA are also required for normal growth and functional development. Interestingly, increased consumption of DHA may also diminish the severity of depression (Hibbeln et al 1995). Children ingesting fish oil more than once a week had a lower probability of suffering from asthma (Hodge et al 1996). Increasing the levels of DHA and EPA in patients with rheumatoid arthritis and ulcerative colitis has also been found to reduce pain and improve conditions, although the modes of operation are unclear at
this point (Stenson et al 1992, Simopoulos 2002). There is currently a large demand for microalgae in the nutraceutical and pharmaceutical industry due to their health-promoting effects. Microalgal derived PUFA, such as ARA and DHA are added as fortifications to infant formulae.

### 1.10.3 Production in Microalgae

Microalgae (single-celled eukaryotic organisms) are the primary natural producers of LC-PUFA. These organisms offer a promising vegetative and non-polluted resource for biotechnology and bioengineering of LC-PUFA production as an alternative to fish oil.

Many marine microalgal strains have oil contents of between 10–50 %, (w/w) and produce a high percentage of total lipids (up to 30–70% of dry weight) (Ward et al 2005). The accumulation of fatty acids is closely linked to microalgal growth stages, functioning as an energy stockpile during unfavorable conditions or cell division. Omega-3 is accumulated due to its high energy content, as well as the good flow properties crucial for cellular functions (Tiez et al 2010, Cohen et al 2000). To date, the omega-3 fatty acid content of numerous microalgae strains have been studied. Strains from the genera *Phaeodactylum, Nannochloropsis, Thraustochytrium* and *Schizochytrium* have demonstrated high accumulation of EPA and/or DHA. *Phaeodactylum tricornutum* (Yongmanitchai et al 1991) and *Nannochloropsis sp.* (Sukenik et al 1991) demonstrated an EPA content of up to 39 % of total fatty acids, while strains such as *Thraustochytrium* (Burja et al 2006) and *Schizochytrium limacinum* (Zhu et al 2007) contained a DHA percentage of between 30–40 % of total fatty acids when grown heterotrophically. High biomass and commercially acceptable EPA and DHA productivities are achieved with microalgae grown in media with optimized carbon & nitrogen concentrations, controlled pH and temperature conditions (Griffiths et al 2009). High oil production, including DHA from *Schizochytrium* (50% w/w),
can be obtained as a result of high growth rate by controlling of nutrients such as glucose, nitrogen, sodium and some other environmental factors, such as oxygen concentrations as well as temperature and pH, achieving high cell densities and DHA productivities (Ward et al 2005).

1.10.4 Biosynthesis in Microalgae

The biosynthesis of omega-3 fatty acids (EPA & DHA) occurs through series of reactions which can be divided into two distinct steps. First is the de novo synthesis of oleic acid (18:1 n-9) from acetate. This is followed by conversion of oleic acid (18:1 n-9) to linoleic acid (LA, 18:2 n-6) and α-linolenic acid (ALA, 18:3 n-3). It was followed by a number of subsequent stepwise desaturation and elongation steps to form an n-3 PUFA family including EPA (Figure 1.6). Nearly all biological systems, including microorganisms, insects, higher plants and animals, are capable of de novo fatty acid synthesis from acetate to short chain fatty acids, with oleic acid (18:1 n-9) as the major product. The biosynthesis starts with the carboxylation of acetyl-CoA to form acetate or pyruvate by the action of glycolytic enzymes. Then acetyl-CoA is converted into malonyl-CoA, which is used to drive a condensation reaction to extend the acyl group to stearic acid (18:0) and desaturate to oleic acid (18:1 n-9).

Oleic acid (18:1 n-9) is further desaturated by a D12 desaturase to form linoleic acid (18:2 n-6) and a D15 desaturase to form α-linolenic acid (18:3 n-3). Then, the n-9, n-6 and n-3 fatty acid families are formed from their precursors by a series of desaturation and elongation reactions. The biosynthesis of the three families of fatty acids are shown in Figure 1.6. The three parent fatty acids-oleic acid, LA and ALA-compete with each other for the D6 desaturase. The affinity of the enzyme to the substrate and the amount of substrate available determine which metabolic pathway is predominant
(Gurr 1985). Generally, the first D6 desaturation is the limiting step and ALA has the highest affinity for D6 desaturase followed by LA and oleic acid.

Most algae, fungi, bacteria, mosses, insects and some invertebrates possess the desaturase and elongase required for the synthesis of various PUFAs. They are the primary producers of these fatty acids in nature (Apt & Behrens 1999). In contrast, higher plants and animals lack the requisite enzymes and, thus, rarely contain PUFAs above C18 (Gill & Valivety 1997).
Figure 1.6 Biosynthesis of three families of polyunsaturated fatty acids (Wen & Chen 2003)
1.10.5 Induction of Omega-3 Production in Autotrophic Microalgae

An increase in microalgal lipid content can be induced by a sudden change of growth conditions. The accumulation of starch and/or lipids reserves is considered to be a survival mechanism in response to growth-limiting stresses (Schenk et al 2008), such as UV radiation (Singh et al 2002), temperature (de Castro et al 2002) and shock or nutrient deprivation (Otero et al 1997, Wen et al 2001), as long as light conditions are present that still allow efficient photosynthesis. For example, during nutritional deprivation (e.g. nitrogen) and under the provision of light, cellular division of many marine or brackish microalgae is put on hold and cells begin to accumulate lipids (Sheehan et al 1998), leading to a 2–3 fold increase in lipid content. Both total lipid and omega-3 fatty acid production can be adjusted by varying growth conditions. The diatom Phaeodactylum tricornutum can be induced to increase its lipid level from 81.2 mg/g of culture dry weight to 168.5 mg/g dry weight (Yongmanitchai et al 1991). Similarly, Nannochloropsis sp. (pal et al 2011) and Dunaliella sp. (Takagi et al 2006) can achieve a total lipid content of up to 47 % and 60 % of dry ash weight by modifying the light intensity, temperature and salinity levels. Omega-3 fatty acid biosynthesis can be stimulated by a number of environmental stresses, such as low temperature, change of salinity or UV radiation. For example, Pavlova lutheri increased its relative EPA content from 20.3 to 30.3 % when the culture temperature was reduced to 15 °C (Tatsuzawa et al 1995). Similarly, Phaeodactylum tricornutum had a higher EPA content when the temperature was reduced from 25 °C to 10 °C for 12 h (Jiang et al 2004). Salinity may also regulate PUFA biosynthesis, but not in a consistent manner. Crythecodinium cohnii has increased its total DHA content up to 56.9 % of total fatty acids when cultured in 9 g/L NaCl. Other treatments that cause the generation of reactive oxygen species and lipid peroxidation also result in higher PUFA contents.
For example, *Phaeodactylum tricornutum* increased its EPA content up to 19.84 % when stressed with UV light (Liang et al 2006).

### 1.10.6 Omega-3 Fatty Acid Production: A Biorefinery Approach

The natural capacity of microalgae to produce multiple products (e.g. oils, proteins and carbohydrates) has encouraged the development of a biorefinery concept for processing. Akin to the petrochemical industry, where crude oil is processed to yield petroleum and a range of other chemicals, microalgae can be processed to produce a range of bioproducts. Different industries are able to use different algal products. For instance, the pharmaceutical and nutraceutical industries use high value bioactive products such as omega-3 fatty acids and carotenoids; the transport industry can use fatty acids from TAG for biodiesel; the chemical industry can use products such as glycerine, while the majority of the biomass can be used by agriculture and aquaculture as animal feed (Subhadra 2011). Additional processes that address nutrient recycling and carbon sequestration can be used by anaerobic digestion of wet biomass and pyrolysis for the production of biochar. Undoubtedly, the biggest interest in microalgal use is for biodiesel production. It potentially represents a more sustainable alternative to fossil fuels as microalgal production facilities do not need to compete for arable land or freshwater. Furthermore, in comparison to land plants, 10–400 times more energy per acre can potentially be produced from microalgae. Although there has been considerable interest and research over the past years into microalgal biofuel production (Sheehan et al 1998), no commercial enterprise has successfully established itself as a supplier of autotrophically derived algal biofuels for any duration. Nevertheless, decreasing fossil fuel reserves and increasing fuel costs continue to drive research targeted towards economically viable production of micro-algal biodiesel, with the level of improvement necessary now appearing attainable (Schenk et al 2008). There is confidence among companies producing microalgae that the production of a
high value product, such as omega-3 from microalgae, will further assist in the establishment of the microalgae industry. Several companies have shifted their focus from algal biodiesel production, to high value products such as omega-3 and protein-rich biomass as animal feed (e.g. Aurora Algae, MBD, Cellana).

1.10.7 Occurrence of Omega-3 in Microalgae

Microalgae have been largely cultured and commercialized as food and feed additives. The ability to positively affect human’s health due to their original chemical composition, is well known. These organisms offer a promising vegetative and non-polluted resource for biotechnology and bioengineering of LC-PUFA production as an alternative to fish oil. Diverse photosynthetic and heterotrophic, mainly marine planktonic species belonging to different classes produce LC-PUFA of the ω-3 family—EPA and DHA (Table 1.1).

Microalgal LC-PUFAs are transferred through food webs, enriching aquatic organisms with these important components. This is especially important in the marine food web because of the marine fish’s limited capacity to synthesize LC-PUFA de novo from the essential LA and ALA.
Table 1.1  LC-PUFA occurrence in various microalgae classes (Guiry & Guiry 2011)

<table>
<thead>
<tr>
<th>Class</th>
<th>Genus species</th>
<th>Major LC-PUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillariophyta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Diatoms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillariophyceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediophyceae</td>
<td><em>Phaeodactylum tricornutum</em></td>
<td>EPA</td>
</tr>
<tr>
<td></td>
<td><em>Nitzschia laevis</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Odontella aurita</em></td>
<td></td>
</tr>
<tr>
<td>Chlorophyta (green algae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prasinophyceae</td>
<td><em>Ostreococcus tauri</em></td>
<td>EPA, DPA</td>
</tr>
<tr>
<td></td>
<td><em>Micromonas pusilla</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pyramimonas cordata</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Parietochloris incise</em></td>
<td>ARA</td>
</tr>
<tr>
<td></td>
<td><em>P. incisa (Δ5 desaturase mutant)</em></td>
<td>DGLA</td>
</tr>
<tr>
<td>Cryptophyta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptophyceae</td>
<td><em>Chroomonas salina</em></td>
<td>DHA</td>
</tr>
<tr>
<td>Dinoflagellata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinophyceae</td>
<td><em>Pyrocystis fusiformis</em></td>
<td>EPA,OPA,SDA</td>
</tr>
<tr>
<td></td>
<td><em>Pyrocystis lunula</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pyrocystis noctiluca</em></td>
<td></td>
</tr>
<tr>
<td>Eustigmatophyta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eustigmatophyceae</td>
<td><em>Nannochloropsis sp.</em></td>
<td>EPA</td>
</tr>
<tr>
<td></td>
<td><em>Nannochloropsis salina</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Nannochloropsis oculata</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Monodus subterraneus</em></td>
<td>EPA</td>
</tr>
</tbody>
</table>
The diversity of microalgal classes with respect to LCPUFA production is illustrated in Table 1.1. The omega-3 LCPUFA EPA and DHA are abundant in representatives of different classes dwelling in marine environments. This capacity is utilized in aquaculture nutrition and fish farming where many microalgal species are routinely cultivated to provide fish with EPA and DHA (Benemann 1992, Lavens & Sorgeloos 1996).

Initially twenty marine microalgae were screened for the production of polyunsaturated fatty acids. Among them, *Isochrysis galbana* and *Nannochloropsis oculata* were selected mainly because of their relatively high growth rate, high lipid content (especially EPA & DHA), resistance to mixing and contamination together with high nutritional values. Their fatty acid profiles are found suitable for incorporation in foods as omega source. Also they are widely cultivated in aquaculture industries and abundantly available.
1.10.8 **Recommendation of Dosage of Omega-3**

Number of countries (Canada, Sweden, United Kingdom, Australia, Japan), as well as the World Health Organization and North Atlantic Treaty Organization have made formal population based dietary recommendations for omega-3 fatty acids. Typical recommendations are 0.3-0.5 g/day of EPA and DHA and 0.8-1.1 g/day of ALA (Welch et al 2006). The Acceptable Macronutrient Distribution Range (AMDR) for ALA is estimated to be 0.6%-1.2% of energy, or 1.3-2.7 g/day on the basis of a 2000-calorie diet which is nearly 10 times the current intake of EPA and DHA (Kris-Etherton et al 2003). These recommendations can easily be achieved by following the American Heart Association Dietary Guidelines, consuming two fish meals per week, with an emphasis on fatty fish (i.e., salmon, herring and mackerel) and using liquid vegetable oils containing ALA (Kris-Etherton et al 2003). In this way, the addition of microalgae biomass cookies and pasta products seems to be an interesting way to increase omega-3 PUFA consumption through an economical and practical product like cookies and pastas. The ingestion of 100 g (0.1 kg) of pasta enriched with biomass of *Isochrysis galbana* (0.5, 1.0 and 2.0 g/100 g) provides 3.1, 6.5 and 11.0%, respectively (Fradique et al 2013), of the recommended daily dose of EPA and DHA (500 mg/day (ISSFAL, 2004)).

1.11 **DEVELOPMENT OF FUNCTIONAL FOODS**

The conception of healthy food has been evolving with time all over the world. Currently, consumers are increasingly making better food choices that provide health benefits. Therefore the role of functional foods has been gaining significant importance. Functional food generally contains one or more functional ingredients that provide an additional health benefit in addition to the energetic and nutritional aspects that every food must confer.
(Plaza et al 2010). Functional food ingredients have biological benefits beyond normal nutrition and have the potential to improve human health. These ingredients are available as dietary supplements which may be incorporated into food products.

The demand for functional foods incorporated with omega-3 fatty acids is increasing over the years due to their added health benefits such as reducing the risk of cardiovascular diseases, type II diabetes, ocular diseases, arthritis etc.

1.11.1 Cookies

Cookies are ready to eat, cheap and convenient food products that are consumed by people of all age groups in many countries and holds an important position in snack food. Cookies were reported to be rich in fat and carbohydrate. Hence they can be referred as energy giving food as well as good sources of Protein and minerals (Opawale et al 2011).

A study was carried out by Gouveia et al (2007) to determine the effects of *Chlorella vulgaris* biomass as a colouring ingredient in traditional butter cookies. The cookies were manufactured at a pilot scale and stored for 3 months at room temperature, protected from light and air. The effects of *C. vulgaris* incorporation on the biscuits' colour were tested weekly during the selected time intervals for a total period of 3 months. *C. vulgaris* cookies presented an accentuated green tonality, which increased with the quantity of added biomass. The colour parameters of the cookies remained very stable throughout the storage period. The texture of the cookies was also evaluated, and a significant increase of their firmness was evidenced with an increase of added microalgal biomass.

Flaxseed flour cookies have been previously prepared by supplementing with wheat flour in various concentrations ranging from 5-
30% by Hussian et al (2006). Composite flours were used to prepare cookies. Cookies prepared without flaxseed flour were kept as control. The mean quality score of the cookies decreased with the increase in the level of the flaxseed flour supplementation. Color and crispiness of the cookies showed a declining trend as compared to flavor and texture of cookies. Cookies containing 20% and lower level of the full fat flaxseed flour were found to be acceptable in relation to their overall acceptability. Significant reduction in the spread factor of the cookies was observed. Addition of the flaxseed flour restricted the spread of the cookies.

Apple fibre flour was added to typical composition of biscuits for the enrichment of nutrients was earlier reported by Kohajdová et al 2011. They have evaluated the effects of commercial apple fibre powder (0, 5, 10 and 15% substitution of fine wheat flour) on dough characteristics, physical and sensory properties of cookies. Addition of apple fibre powder significantly altered rheological parameters of dough by increasing the water absorption, dough development time and dough stability, however mixing Tolerance index was reduced. Apple fibre powder incorporation to cookies negatively affected specific volume, volume index of cookies, reduced thickness and width of products. It also revealed that the apple fibre powder addition resulted in notable decrement of lightness parameter and increment of redness in cookies. Sensory evaluation showed that cookies with apple fibre powder were characterized with sweet fruity taste/odor and were firmer than samples without apple fibre. Addition of apple fibre powder up to 10% is feasible to produce sensorially acceptable cookies. Finally it has been concluded that the enrichment of cookies with apple fibre powder is advantageous due to the increased nutritional value, as apple fibre is rich source of dietary fibre.

Traditional butter biscuits were enriched with *Isochrysis galbana* biomass (1 and 3%) and evaluated in terms of color, texture and fatty acid profile, for storage period of 3 months (Gouveia et al 2008). *Isochrysis*
galbana biscuits presented total levels of 100 and 320 mg/100g of PUFA-ω3 (EPA+DPA (docosapentaenoic acid; 22:5ω3)+DHA (docosahexaenoic acid; 22:6ω3) for 1 and 3 % of biomass, respectively. Eventually they have enhanced good texture properties, high stability of color, texture and good profile of polyunsaturated fatty acids, with emphasis on EPA and DHA.

Nutritional, sensory and textural characteristics of defatted mustard flour fortified biscuits were studied (Tyagi 2007) to optimize the mustard flour supplement in the blend for making biscuits. The wheat flour was replaced by defatted mustard flour at 5, 10, 15 and 20 % incorporation levels in biscuit preparation. The protein content of mustard flour biscuit increased nearly 2.5 times as a result of mustard flour incorporation, coupled with reduction in fat and an increase in fiber content. Sensory evaluation results revealed that the sample containing 15 % defatted mustard flour scored highest in most of the attributes including the overall acceptability. Textural characteristics of all dough and biscuit up to 15 % supplement of defatted mustard flour were similar while at 20 % level, the values were significantly different. The study reveals that incorporation of 15 % defatted mustard flour gave desirable results in terms of nutritional, sensory and textural attributes of mustard fortified biscuits.

1.11.2 Pasta

Pasta has a good nutritional profile, providing a source of complex carbohydrates, and it has good consumer value. It has relatively long shelf life, if stored in proper conditions. It is easy to cook and can be employed in variety of meals (Marchylo & Dexter 2001).

Pasta products such as macaroni, spaghetti, vermicelli and noodles are manufactured from durum wheat (Triticum durum), known to be the best raw material suitable for pasta production due to its unique colour, flavour and cooking quality (Feillet & Dexter 1996). In recent years, pasta has become
more popular due to its acceptability among all age groups, low cost, convenience of transportation, long shelf life and nutritional properties, being regarded as a product with low glycaemic index. Pasta is low in sodium, lipids and it has no cholesterol producing a low-postprandial response to glucose and insulin in the blood (Cleary & Brennan 2006, Tudorica et al 2002). Nutritional quality of pasta can be enhanced through the addition of non-traditional raw materials rich in fibres (Chillo et al 2008), vitamins and polyunsaturated fatty acids (Verardo et al 2009).

Japan plays an important role in the utilization of microalgae and there has also been a recent upsurge in research and development on the utilization of microalgae as an ingredient and source of a wide range of metabolites such as bioactive compounds, pigments, and essential fatty acids. Few studies have been carried out concerning incorporating seaweed into pasta products: Chang and Wu 2008 have studied the potential of green seaweed (*Monostroma nitidum*) powder in oriental fresh egg noodles; while Prabhasankar et al (2009) have evaluated the effect of different levels of brown seaweed *Undaria pinnatifida* on the sensory, cooking, nutritional and biofunctional quality of pasta. Recently, there are numerous commercial applications for microalgae. For example, microalgae can be used to enhance the nutritional value of food and animal feed due to their chemical composition. They also play a crucial role in aquaculture and they can be incorporated into cosmetics. Moreover, they are cultivated as a source of highly valuable molecules. For example, polyunsaturated fatty acids are added to infant formulas and nutritional supplements and pigments are important as natural dyes (Becker 2004).

Pasta enriched with different amount of microalgae biomass (*Chlorella vulgaris* and *Spirulina maxima*) was prepared by Fradique et al (2010). The incorporation of microalgae resulted in an increase of quality parameters when compared to the control sample. The colour of microalgae pastas
remained relatively stable after cooking. The addition of microalgae resulted in an increase in the raw pasta firmness when compared to the control sample. Of all the microalgae studied, an increase in the biomass concentration (0.5–2.0%) resulted in a general tendency of an increase in the pasta firmness. Sensory analysis revealed that microalgae pastas had higher acceptance scores by the panellists than the control pasta. Microalgae pastas presented very appellative colours (orange and green), nutritional advantages and energetic values similar to pastas produced with vegetables. The use of microalgae biomass enhanced the nutritional and sensorial quality of pasta, without affecting its cooking and textural properties.

Pasta enriched with different amounts of Isochrysis galbana (Ig) and Diacronema vlkianum (Dv) biomass was prepared as a source of Polyunsaturated fatty acids (PUFA) (Fradique et al 2013). The results showed that fatty acid profile of pastas prepared with Ig and Dv biomass incorporation, presented a high resistance to the thermal treatment applied during the cooking procedure. The ingestion of 100 g (0.1 kg) of pastas enriched with biomass of Ig (0.5, 1.0 and 2.0 g/100 g) provides 3.1, 6.5 and 11.0 %, respectively, of the recommended daily dose of EPA and DHA (500 mg/day (ISSFAL, 2004)), while the pastas with Dv biomass provides 0.5-2.0%. These results confirmed the importance of microalgae incorporation in traditional foods to enrich their nutritional value by bioactive molecules. The sensorial evaluation revealed a slight depreciative fish odour for the pasta with higher microalgal biomass contents (2 g/ 100 g).

1.11.3 Chikkis

Chikki is one of the popular and traditional ready-to-eat Indian snack. It is generally made from roasted peanuts (Arachis hypogaea) and jaggery. It is a very popular sweet item in both rural and urban South Asia (spanning India, Pakistan, Bangladesh, Nepal and Sri Lanka). It is popularly known as
Peanut brittle in western countries. Jaggery is a concentrated product of date, cane juice or palm sap without separation of molasses and crystals. It contains proteins, minerals and vitamins and a potent source of iron and copper (Manay & Swamy 2001). There are different varieties of chikkis in addition to the most common peanut chikki. Usually, ingredients such as puffed or roasted bengal gram, sesame, puffed rice, beaten rice and khobara (desiccated coconut) are used (Chetana & Sunkireddy 2011). It has a relatively long shelf life, if properly stored. Because of their acceptability among all age groups, longer shelf life and better taste, they are considered as a good product for omega-3 fatty acid fortification and nutritional improvement.

In an attempt to achieve correct ratio of n-3 to n-6 fatty acids for health benefits, flaxseed (*Linum usitatissimum*) incorporated chikkis were prepared by Chetana & Sunkireddy 2011. Tertiarybutylhydroquinone (TBHQ) at 200 ppm level used as an antioxidant. Results showed that there were no differences in texture or sensory quality among the samples with and without addition of antioxidant. The peroxide value of oil in chikki increased gradually at 37 °C during storage. At the end of 60 days at 37 °C, rancidity developed in the samples without antioxidant but not in the one with added antioxidant. Thus, TBHQ increased the shelf-life of the product. Addition of flaxseeds to chikki increased PUFA content, especially n-3 fatty acids, up to 9 %, which were not present in chikki prepared only with peanuts. Thus the ratio of 18:2 to 18:3 increased with addition of flaxseed, which has significant health benefits.

1.12 MICROENCAPSULATION

Microencapsulation (ME) has been defined as ‘the technology of packaging solid, liquid and gaseous materials in small capsules that release their contents at controlled rates over prolonged periods of time’.
Microencapsulation is one of the strategies used by industry to protect the polyunsaturated fatty acids of external factors which initiate the oxidation process that can produce off-flavors, both during processing and storage. It is also used to mask any unwanted odor and flavor in the final product and to facilitate handling (Kolanowski & Laufenberg 2006).

1.12.1 Microencapsulation by Spray Drying

Omega-3 and omega-6 fatty acids are used for food fortification having considerable health benefits. However, major issues have been encountered with regards the taste and smell of these oils and their propensity to oxidize rapidly (Augustin & Sanguansri 2003). It was demonstrated that the consumption of food enriched with microencapsulated fish oil (emulsion spray-drying) was as effective as the daily intake of fish oil gelatin capsules in fulfilling the dietary requirements of this omega-3 long-chain fatty acid (Wallace et al 2000).

The process involves four stages such as preparation of a dispersion or emulsion, homogenization of the dispersion, atomization of the feed emulsion and dehydration of the atomized particles (Shahidi & Han 1993). Many different inlet/outlet temperature combinations are used, in most of the cases based on core material, wall material, addition ratios and equipment capacity. Typical inlet/outlet temperatures used in research for microencapsulation include 180/70 ºC, 160/60 ºC and 210/90 ºC for n-octenylsuccinate-derivatized starch and glucose syrup coated fish oil (Serfert et al 2009, Drusch & Berg 2008), 210/95 ºC for refined menhaden oil (Baik et al 2004) and 180/80 ºC for avocado oil.

The effectiveness of spray-drying is critically dependant on the type of wall material used. For spray drying to yield powder with high quality attributes, the emulsion should have the following properties: small size and narrow distribution of oil droplets stable to agglomeration and coalescence, a
high solid content for enabling formation of a continuous matrix as a protective barrier where oil droplets are uniformly distributed and embedded, a low viscosity for easy flow, pump and spray, and a proper ratio of wall and oil materials (Bae & Lee 2008). Important attributes evaluated in spray dried encapsulated oil powders are: regular shape, surface oil content, microencapsulation efficiency, moisture content, oxidative stability, particle size, wettability, flowability and color. Emulsification and spray drying are used to have a composition of 25-30% oil in matrix (Barrier & Rousseau 1998) and particle size ranging from 10-100 μm (Könstanc et al 1995). According to Drusch & Berg (2008), depending on the extraction method used, oil load, and spray drying conditions, the non-encapsulated oil (% of the total oil) of fish oil microcapsules ranges from 0.99 ± 0.03 to 13.5%.

Carbohydrates, proteins, and gums are the most commonly used wall materials for spray drying. Among these, gum arabic, modified starches including n-octyl succinate starch and maltodextrins, whey and soy protein, gelatin, sodium caseinate, alginate, carrageenan, and pectin are most commonly used (Gharsallaoui et al 2007, Jin et al 2008).

For freeze drying the emulsion is frozen at temperature between −90 and −40 °C and then dried by sublimation under low pressure. The main advantage of this technology is the removal of oxygen and the application of low temperatures, which help minimize product oxidation. This commonly leads to good rehydration performance of the powdered product (Stapley 2008). These advantages of freeze drying make this technology suitable for the microencapsulation of highly sensitive ingredients, such as probiotics and PUFAs. Few studies have explored the microencapsulation of omega-3 fatty acids using freeze drying. Heinzelmann et al.(2000) reported that freeze drying of microencapsulated fish oil gives powders with highly porous structures and a short shelf-life. However, FD method involved low temperature requirements, maintaining high vacuum and long residence time.
that leads to high capital and operating costs. Furthermore, it was found that
the rate controlling step of FD is the diffusion of water through the solid, thus
reducing the particle size which results in increased quickness of process
(Malecki et al. 1970). Moreover, the drying times are varying approximately
with the square of the sample thickness (as per Pharm equation), thus reduce
the dimensions of the material reduce the drying time in a great extent (Al-
Hakim & Stapley 2004).

Whey protein and maltodextrin are two of the most commonly studied
encapsulated tuna oil in whey protein combined with chitosan and
maltodextrin by ultrasonic atomization. The process produced microcapsules
with good encapsulation efficiency (~80%) and with little loss of DHA and
EPA following processing.

Whey protein isolate (WPI) also has been used to encapsulate flaxseed
oil, a good source of α-linolenic acid (ALA, 18:3) (Partanen & Raula 2008).
Microcapsules were prepared by spray drying. WPI was used at a level of
10 % (w/w) and flax oil was added at 40 % of WPI content. Comparing
oxidation of bulk flax oil to the microencapsulated oil showed that the
oxidation rates were similar for three weeks of storage at 37 °C after which
oxidation of bulk oil proceeded more rapidly.

Rusli & Sanguansri (2006) compared WPI to soy protein isolate (SPI)
in combination with protein-glucose syrups for microencapsulation of fish oil
by spray drying. Protein: carbohydrate ratios were maintained at 1:2 while oil:
protein ratios were varied from 0.75:1 to 4.5:1. These authors also evaluated
effects of oil composition by encapsulating tuna oil, palm stearin oil and tuna
oil blended with palm stearin oil. Drying temperatures were 180 and 80 °C.
The emulsion was formed using a two stage homogenizer with different
pressures evaluated. Increasing oil content did not cause an increase in oil
droplet size for whey protein emulsions; however, emulsions prepared with SPI and oil: protein ratios of 3:1 and 4.5:1 displayed evidence of coalescence. Oil droplet sizes ranged from 0.25-0.5 μm. Higher homogenization pressures produced emulsions with smaller oil droplet sizes. Encapsulation efficiencies of WPI microcapsules were 97 % at the lowest oil: protein ratio and 86 % at the highest. SPI microcapsules had encapsulation efficiencies of 93 % for the lowest ratio and 81 % for the highest. The better performance of WPI was attributed to better film forming properties than SPI.

Shaw et al (2007) investigated the stability of spray dried tuna oil in water emulsions prepared with lecithin, chitosan and corn syrup solids. Emulsions were prepared above and corn syrup solids were added at varying levels for spray drying. Spray drying was carried out at an inlet temperature of 180 °C on a spray drier with a rotary disk atomizer. Oxidative stability was evaluated during storage at 37 °C and 33 % RH. The authors found that powders encapsulated with 5 % (w/w) corn syrup solids had the greatest oxidative stability relative to those prepared with 1, 2, 10 and 20 %, but that the physical stability of the powders was better for those prepared with 10 % or 20 % corn syrup solids. Particles prepared with only 1 or 2 % corn syrup solids exhibited undesirable agglomeration of particles suggesting these levels of corn syrup solids were not sufficient to produce usable microcapsules.

Microencapsulation of flax oil was investigated using zein as the coating material by Quispe-Condori et al (2011). Central Composite Design-Face Centered was used to optimize the microencapsulation with respect to zein concentration (x1) and flax oil concentration (x2) using spray drying. The quality of microcapsules was evaluated by determining encapsulation efficiency, flowing properties (Hausner ratio), and evaluating the morphology with scanning electron microscopy. The response surface model for microencapsulation efficiency showed a high coefficient of determination (R2 ¼ 0.992) and a non-significant lack of fit (p ¼ 0.256). The maximum
microencapsulation efficiencies were 93.26±0.95 and 59.63±0.36 % for spray drying and freeze drying, respectively. However, microcapsules prepared by spray and freeze drying had very poor handling properties based on the Hausner ratio. The bulk density decreased with an increase in zein concentration at the same flax oil concentration. The morphology of the flax oil microcapsules depended on the zein:flax oil ratio and the process used for microencapsulation. Flax oil microcapsules prepared by spray drying appeared to be composed of heterogeneous spheres of various sizes at high zein:flax oil ratios. Microcapsules prepared by freeze drying resulted in agglomerated small spheres. These microcapsules might find a niche as functional food ingredients.

Umesha et al (2012) prepared microencapsulated Green cress seed oil (MGCO) using whey protein concentrate with oil/protein ratio of 0.4, by spray-drying method. Microencapsulated GCO powder (MGCO) contained 25 g of GCO/100 g with microencapsulation efficiency of 64.8% and particle size of 15.4 to 9.1 microns. Biscuits were prepared by supplementing MGCO at 20 g/100 g or GCO at 5.0 g/100 g by replacing flour and fat or fat in biscuit formula. ALA content was found to be 1.02 g and 1.05 g/100 g respectively in MGCO and GCO supplemented biscuits. Biscuits were packed in metalized PET film (MPET) pouches, stored at three different storage conditions, viz., 90% RH/38 °C for 3 months, 30-40 % RH/38-40 °C for 4 months and 65% RH/27 °C for 5 months. Biscuits stored at 90% RH/38 °C had one month shelf- life, whereas at 30-40 % RH/ 38-40 °C and 65 % RH/27 °C, they lasted 4 and 5 months, respectively.
1.13 OBJECTIVES OF THE STUDY

Current sources of PUFA are from marine fatty fish (salmon, mullet and mackerel) (Gunstone 1996). Declining fish stocks, fishy odor, seasonal variations in quality also the presence of pollutants such as heavy metals and PCBs in fish (Domingo & Bocio 2007) may leads to health problems. Therefore, other dietary sources of EPA and DHA are being sought. In addition, fish oil is not favourable for vegetarians and the odour makes it unattractive. Food enrichment is probably the best long-term solution to boost intake of long chain ω-3 PUFA (Molendi-Coste et al. 2011). Microalgae are the primary producers of PUFAs in the trophic chain (Barclay et al. 1994). This favors production of EPA and DHA from microalgae. So far the studies are focused on the development of functional foods by using fish oil and microalgal biomass. The direct incorporation of biomass was found to be unacceptable in terms of taste and odour. So in this study we have developed a novel method for production of functional food by direct incorporation of microalgal oil. This can be achieved by the following objectives

1) To study the effect of different light intensities and photoperiod regimes (exposure to light/dark cycles) on cell growth rate, biomass and lipid productivity of *Isochrysis galbana*

2) To study the effect of different parameters (carbon, nitrogen, light intensity and photoperiod) on biomass production and lipid productivity of *Nannochloropsis oculata*.

3) Functional food product development using *Nannochloropsis oculata* and *Isochrysis galabana* as a natural source of Omega 3 fatty acids.