

# Microalgae as a potential source of single-cell proteins. A review

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**Introduction.** Microalgae can be considered as one of the most reliable natural sources of nutrients and other valuable substances that could satisfy the growing needs in food and energy. In this review article, the global potential for the use of microalgae and the protein content of a number of microalgae is examined with a brief description of the microalgae species.

**Literature.** Despite the high protein content and the presence of other valuable substances in microalgal biomass, microalgae mass production is still at its embryonic level. Microalgal proteins exhibit good nutritional, functional and technofunctional properties when compared to some conventional protein concentrates.

**Conclusions.** There is therefore a need for the mass production of microalgae to be boosted by public and private institutions in order to avoid protein shortages.

**Keywords.** Nutrients, protein content, biomass, production.

## Les microalgues comme une source potentielle de protéines d'origine unicellulaire (synthèse bibliographique)

**Introduction.** Les microalgues peuvent être considérées comme une des sources fiables de nutriments et d'autres substances de valeur pouvant satisfaire le besoin croissant en aliment et en énergie. Dans la présente revue, le potentiel global en microalgues, la teneur en protéines et une brève description de certaines espèces de microalgues sont présentés.

**Littérature.** Malgré leur teneur élevée en protéines et en d'autres substances de valeur, la production industrielle des microalgues est encore à l'état embryonnaire. Les protéines des microalgues présentent de bonnes propriétés nutritionnelles, fonctionnelles et technofonctionnelles lorsqu'elles sont comparées aux protéines conventionnelles.

**Conclusions.** La production des microalgues doit donc être encouragée par les autorités publiques et privées afin d'éviter les pénuries de protéines.

**Mots-clés.** Nutriments, teneur en protéines, biomasse, production.

## 1. INTRODUCTION

The search for new protein sources to supplement the existing conventional sources in order to fill the so called "protein gap" has been the route of inspiration for many scientists for years.

Single-cell protein (SCP) refers to protein extracted from pure or mixed cultures of algae, yeast, fungi or bacteria used as a substitute for the conventional protein sources exploited for human and animal consumption. Proteins are macromolecules with a complex chemical structure, and this complexity is the basis of their multiple physiological, morphological, and technological uses. Proteins can be used as sole protein concentrates or may be integrated into processed foods. In the latter case, each constituent of the processed food product plays a specific role, be it nutritional, technological or functional. Knowledge

of the technofunctional and nutritional properties of proteins is therefore a prerequisite for their proper utilization in the food industry.

Microalgae have been identified as one of the most reliable sources of protein and were a source of interest to the majority of those involved in agricultural and food domains during the second half of the twentieth century. Some microalgal sources present a protein content higher than conventional animal or plant sources, e.g. the protein content of *Spirulina paltensis* is 65% higher than that of dried skimmed milk (36%), soy flour (37%), chicken (24%), fish (24%), beef (22%) and peanuts (26%) (Moorhead et al., 2011). Despite the tremendous variety of virtuous nutraceutical uses reported for substances derived from algal biomass (Simpore et al., 2006; Yamani et al., 2009), in addition to its high protein content, there is still much to be done in terms

of algacultural implementation in order to make this foodstuff available at an affordable cost.

Of the more than 30,000 microalgae species, fewer than 10 are commercially produced (Gouveia et al., 2008). Despite the fact that some of these microalgae species have been historically used as food, their industrial production is not up to the level that might be expected.

In this review, emphasis will be placed on some of the microalgae species that are currently being industrially produced and/or those that present a relatively high protein content. The global microalgal potential, the protein content of some microalgae species, and the technofunctional and nutritional properties of these algal proteins will be presented, as reported in the literature.

## 2. GLOBAL MICROALGAE PRODUCTION POTENTIAL

Microalgae (unicellular organisms) and macroalgae (multicellular organisms) belong to the large algae group made up of photosynthetic organisms. They are known to have appeared on Earth 3.5 billion years ago and they are considered to be the first form of life (Margulis, 1981). Like terrestrial plants, algae are autotrophic organisms; however, they lack stems, leaves, flowers, and are rootless. Among the microalgae, while some are eukaryotic and commonly identified as algae, others are devoid of a membrane-bound nucleus (prokaryotes, cyanobacteria) and bear an intermediate structure between bacteria and plants. Microalgae have a higher level of productivity than traditional crops and can be grown in climatic conditions, such as desert and coastal areas (Christaki et al., 2011). Their production yield is not only high but also environmentally friendly. Marshall<sup>1</sup> (2007) cited by Christaki et al. (2012) reported that microalgae are the most productive sources of nutrients in the world. There is growing interest in microalgae, due to their ability to concentrate essential nutrients and functional substances.

Microalgae production initially mistakenly focused its marketing efforts on health foods, which represent a small market and cannot instigate a large demand. This orientation has been found to be responsible for the limited development of the industrial mass production of microalgae (Richmond, 2004). Yet, many microalgae (such as *Spirulina*, *Chlorella*, *Dunaliella*, *Scenedesmus*), when correctly processed, have an attractive taste and could thus be incorporated into many types of food. This would lead to an increase

in the demand for microalgae (Richmond, 2004). The daily production rate of protein-rich microalgae cell mass presents an annual yield of some 250 t·ha<sup>-1</sup>, *i.e.* several times that of any agricultural commodity (Richmond, 2004).

Mass production of microalgae can be carried out outdoors or in bioreactors, under optimal conditions. Microalgae provide an opportunity to exploit the underutilized arable lands and oceans without reducing the agricultural production surface, which needs to be supplemented in order to feed the growing global population. The use of microalgae in waste water treatment, for biofuel production and for atmospheric carbon dioxide sequestration (owing to their photosynthetic activities) is also boosting the demand for mass production.

*Chlorella* is among the most ancient microalgae species used in the human diet and its commercial utilization was introduced in 1961 by Nihon Chlorella Inc. in Japan (Iwamoto, 2004). *Chlorella* was initially cultivated for its use as a health food (it contains  $\beta$ -1, 3-glucan, an immunoactive substance) and then in mariculture. The total amount produced in the 1990s was 2,000 t per year (Iwamoto, 2004). The mass production mode for *Chlorella* is either heterotrophic or mixotrophic. Production yield is higher in the latter production mode. In the mixotrophic production, acetic acid can be added to the medium as an organic carbon source in addition to the carbon dioxide. In the heterotrophic mode, carbon is supplied by the sole organic carbon source. However, *Chlorella* biomass obtained by heterotrophic mass production exhibits a superior quality for consumption as a health food; it is rich in valuable phytochemicals and does not present contaminants (Iwamoto, 2004). A study carried out by Praveenkumar et al. (2014) on *Chlorella* sp. modes of mass production (autotrophic and mixotrophic) revealed that a mixotrophic fed-batch cultivation with glucose and a supply of air in dark cycles showed the highest levels of biomass (561 mg·l<sup>-1</sup>·d<sup>-1</sup>) and fatty-acid methyl-ester (168 mg·l<sup>-1</sup>·d<sup>-1</sup>) productivity.

*Arthrospira* (*Spirulina*) sp. also has an historical background in human consumption. *Spirulina maxima* is recorded as having been used for human consumption, in dried cake form since 1521, in Tenochtitlan (modern-day Mexico City) (Hu, 2004). The first commercial mass production of *Spirulina* began in the 1970s in Lake Texcoco. The use of *Spirulina* in the human diet also has a long history among the Kanembu tribes living around Lake Chad in Chad Republic. However, it remained unnoticed until the 1960s. *Spirulina* sp. is considered to be an obligatory alkalophile, with the maximal growth rate being obtained at pH 9.5-9.8 (Hu, 2004). Its ability to thrive in a high pH environment limits the development of other microorganisms and favors its large-scale outdoor mass production. Annual

<sup>1</sup> Marshall H., 2007. Micro-algae as a superfood source: phytoplankton for future nutrition. *Vegetarian*, 1-2.

production of *Spirulina* worldwide was 2,000 t in the year 2000 (Hu, 2004). Mixotrophic mass production presents higher yields (Chen et al., 1997). Today, *Arthrospira* sp. mass production is occurring all over the world, with most of the production facilities being located in the Asia-Pacific region (Lee, 1997).

*Dunaliella* sp. is the most halotolerant eukaryotic photosynthetic organism, known for its remarkable ability to adapt to different salt concentrations from as low as 0.1 M to salt saturation (4 M) (Ben-Amotz, 2004). It is the most suitable organism for outdoor mass cultivation in open ponds. The autotrophic mass production mode is adapted for large-scale *Dunaliella* production in media containing inorganic nutrients with carbon dioxide as the sole carbon source (Ben-Amotz, 2004).

Attempts at the heterotrophic production of *Dunaliella* have not, however, been successful as yet. This organism is one of the best natural sources of  $\beta$ -carotene, and a modern intensive plant of 50,000 m<sup>2</sup> can produce 3,650 kg per year. The optimal growth medium for *Dunaliella* should ideally contain around 1.5 M NaCl, more than 0.4 M MgSO<sub>4</sub>, and 0.1 M CaCl<sub>2</sub> under pH control (Ben-Amotz, 2004).

*Aphanizomenon* sp., a fresh water cyanobacterium was first exploited in the 1980s after the first harvest of natural bloom in Klamath Lake (Oregon, USA) (Carmichael et al., 2000). Two thousand tons of this organism were harvested in 1998 from the lake. Natural harvesting presents some obstacles such as the harmful chemical composition of the harvested biomass and contamination by other species or strains that produce neurotoxins or other undesirable substances. The development of large-scale production photobioreactors for *Aphanizomenon* is therefore of the utmost importance.

*Nostoc* sp. can develop under various climatic conditions including the Polar Regions, hot springs and deserts. The optimum temperature for the growth of this cyanobacterium is between 15 °C and 25 °C (Cui, 1983). It shows a great adaptability to a wide range of temperatures. *Nostoc* was consumed by the Chinese in order to survive during times of famine 2,000 years ago (Danxiang et al., 2004). However, the cultivation of this organism has never progressed beyond the experimental level.

In addition to its relatively high protein content, *Scenedesmus* sp. is mostly cultivated for biofuel production because of its high lipid content (31.7%) and high biomass productivity compared to other microalgae sources (Xia et al., 2014). A recent study carried out in China by Xia et al. (2014) showed that *Scenedesmus obtusus* presented the highest biomass productivity (20.2 g·m<sup>-2</sup>·d<sup>-1</sup>) compared to the other tested species. Microalgae species such as *Scenedesmus* sp. and *Chlorella* sp. are currently mostly being produced

for biofuel synthesis, and the by-product obtained after lipid extraction can be readily valorized for its high protein content.

In another recent study on *Scenedesmus* mass production by Abomohra et al. (2014), *Scenedesmus* sp. was cultivated in a semi-continuous culture for 3 months in polyethylene transparent bags. A maximum productivity of 0.14 g·l<sup>-1</sup>·d<sup>-1</sup> was obtained from this experimental mass production. As part of this study, harvesting methods were also investigated using different flocculants, and a maximum flocculation of 82% was achieved using 250 mg·l<sup>-1</sup> of NaOH for 2 h.

Investigations into the feasibility of growing *Porphyridium* biomass outdoors were carried out as early as 1985 by Vonshak et al. in a laboratory study. It was found that, although the optimum temperature for the growth of *Porphyridium* is known to be 25 °C, no damage to photosynthetic activity was detected after exposure of the organism to higher temperatures, up to 35 °C. In addition, high O<sub>2</sub> evolution activity was observed at relatively high cell concentrations, and no inhibition of O<sub>2</sub> evolution was detected at high light intensity. A production rate of up to 22 g·m<sup>-2</sup>·d<sup>-1</sup> (dry weight) was obtained for several weeks during outdoor cultivation.

In a study on the strain *Porphyridium purpureum* by Velea et al. (2011), the growth rate was optimized using a two-variable experimental design (light and sodium bicarbonate feeding), by amending the ASW (artificial sea water) nutrient medium with additional amounts of NaHCO<sub>3</sub>. The study showed that increased light intensity and NaHCO<sub>3</sub> in ASW medium led to substantial increases in biomass production, as well as in exopolysaccharide yields. Exopolysaccharides and phycobiliproteins are often the targeted substances in *Porphyridium* sp. mass cultivation.

The biotechnology involved in *Porphyridium* outdoor production was developed as early as the late 1970s by Gudin et al.<sup>2</sup> (1991), cited by Arad et al. (2004). Indoor bioreactors for the mass production of *Porphyridium* sp. showed that a custom-built flat-sided photobioreactor with a higher exposed surface area to volume ratio provided the best production system (Iqbal et al., 1993).

Moreno et al. (2003) investigated outdoor mass cultivation of the nitrogen-fixing marine cyanobacterium *Anabaena* sp. ATCC 33047. The authors found that, in open ponds operated under a semi-continuous biomass regime, productivity values achieved ranged from 9 g·m<sup>-2</sup>·d<sup>-1</sup> (dry weight), in winter, to over 20 g·m<sup>-2</sup>·d<sup>-1</sup>, in summer, provided that key operation parameters, including cell density,

<sup>2</sup> Gudin C. & Chaumont D., 1991. Cell fragility – the key problem of microalgae mass production in closed photobioreactors. *Bioresour. Technol.*, **38**, 145-151.

were optimized. Under these conditions, the harvested biomass was rich in high-value phycobiliproteins, namely allophycocyanin and phycoerythrin, for which open cultures of marine *Anabaena* represent a most interesting production system.

An assessment of the CO<sub>2</sub> fixation and biomass productivity of *Anabaena* sp. ATCC 33047 was carried out by Clares et al. (2014). In this study, the highest values achieved for CO<sub>2</sub> fixation rate and biomass productivity were 1 and 0.6 g·l<sup>-1</sup>·d<sup>-1</sup>, respectively.

González López et al. (2009) investigated the carbon dioxide removal capacity of *Anabaena* sp. Results showed a maximum CO<sub>2</sub> fixation rate of 1.45 g·l<sup>-1</sup>·d<sup>-1</sup> CO<sub>2</sub>, which could be increased to up to 3.0 g·l<sup>-1</sup>·d<sup>-1</sup> CO<sub>2</sub> outdoors for the species investigated.

*Tetraselmis* sp. is also a halotolerant form of microalgae that can be used either as food or feed, or for biofuel synthesis. A number of studies have been carried out to assess and improve the mass production conditions for this organism. The ability of *Tetraselmis* sp. to thrive in high salt concentrations is sustained through variable levels of starch production as a result

of metabolic stress linked to new growing media conditions. A study carried out by Yao et al. (2013) on *Tetraselmis subcordiformis* showed that decreased salinity combined with nitrogen-generated moderate stress facilitated starch accumulation. The authors concluded that salinity manipulation can be effectively applied for enhanced starch production in marine microalgae.

Results of a recent pilot-scale mass production study by Fon Sing et al. (2014) showed that a peak productivity of 37.5 ± 3.1 g·m<sup>-2</sup>·d<sup>-1</sup> ash free dry weight (AFDW) was reached in a recycled medium upon transition from 14% to 7% NaCl. The combination of high biomass-yielding mixotrophic growth under high salinity has proved to be a successful sustainable cultivation strategy.

### 3. PROTEIN CONTENT OF SOME MICROALGAE BIOMASS

Most microalgae exhibit a high protein content (**Table 1**), with the largest values being reported for

**Table 1.** Protein content (% dry weight basis), class, and kingdom of different microalgae — *Teneur en protéines (% en masse de matière sèche), classe et règne de différentes microalgues.*

Alga	Protein content (%)	Class	Domain (kingdom)	Reference
<i>Anabaena cylindrica</i>	43-56	Cyanophyceae	Procaryota (Eubacteria)	Becker, 2007
<i>Aphanizomenon flos-aquae</i>	62	Cyanophyceae	Procaryota (Eubacteria)	Becker, 2007
<i>Arthrospira maxima</i>	56-77	Cyanophyceae	Procaryota (Bacteria)	Paoletti et al., 1980
<i>Chlorella ellipsoidea</i>	42.2	Trebouxiophyceae	Eukaryota (Plantae)	Servaites et al., 2012
<i>Chlorella ovalis</i>	10.97	Trebouxiophyceae	Eukaryota (Plantae)	Slocombe et al., 2013
<i>Chlorella pyrenoidosa</i>	57	Trebouxiophyceae	Eukaryota (Plantae)	Becker, 2007
<i>Chlorella spaerckii</i>	6.87	Trebouxiophyceae	Eukaryota (Plantae)	Slocombe et al., 2013
<i>Chlorella vulgaris</i>	51-58 53.3	Trebouxiophyceae	Eukaryota (Plantae)	Becker, 2007 Sean et al., 2015
<i>Dunaliella primolecta</i>	12.26	Chlorophyceae	Eukaryota (Plantae)	Slocombe et al., 2013
<i>Dunaliella salina</i>	57	Chlorophyceae	Eukaryota (Plantae)	Becker, 2007
<i>Dunaliella tertiolecta</i>	11.4	Chlorophyceae	Eukaryota (Plantae)	Barbarino et al., 2005
<i>Porphyridium aerugineum</i>	31.6	Porphyridiophyceae	Eukaryota (Plantae)	Sean et al., 2015
<i>Porphyridium cruentum</i>	35 28-39	Porphyridiophyceae	Eukaryota (Plantae)	González López et al., 2010 Becker, 2007
<i>Scenedesmus almeriensis</i>	41.8	Chlorophyceae	Eukaryota (Plantae)	Romero et al., 2012
<i>Scenedesmus obliquus</i>	48 50-55	Chlorophyceae	Eukaryota (Plantae)	González López et al., 2010 Becker, 2007
<i>Spirulina platensis</i>	60-71 55.8	Cyanophyceae	Procaryota (Bacteria)	Paoletti et al., 1980 Sean et al., 2015
<i>Tetraselmis</i>	36	Prasinophyceae	Eukaryota (Plantae)	Schwenzfeier et al., 2011
<i>Tetraselmis chuii</i>	31 46.5	Prasinophyceae Prasinophyceae	Eukaryota (Plantae) Eukaryota (Plantae)	Brown, 1991 Sean et al., 2015

the *Spirulina* (*Arthrospira*) species (55.8 to 77%). The variability within values for protein content reported by different authors is remarkable for some species such as *Dunaliella* sp. This can be seen, for example, in a comparison of the values found for the species *Dunaliella tertiolecta* (11.4%) (Barbarino et al., 2005) and for *Dunaliella salina* (57%) (Becker, 2007) (**Table 1**). Reported values may also depend on the analytical methods used in a study and on the origin of the analyzed biomass (growth medium, harvesting period, production method, etc.).

*Spirulina* sp. (*Arthrospira* sp.) is a genus of free-floating filamentous cyanobacteria characterized by bicylindrical, multicellular trichomes in an open left-hand helix. It is a ubiquitous microalga, capable of adaptation to very different habitats, colonizing certain environments in which life proves very difficult or impossible for other microorganisms (Orio, 1983).

A French phycologist Dangeard (1940) reported that a substance called *Dihé* in the local language (Kanembu), was eaten by the native population, and was obtained by sun drying mats of microscopic algae harvested from the surface of small lakes or ponds around Lake Chad (Orio, 1983). The alga was identified as *Spirulina* (*Arthrospira*) *platensis*. At the same time, another *Spirulina* sp., *S. maxima*, was discovered to be growing abundantly in Lake Texcoco, near Mexico City (Orio, 1983). Findings in the literature have clearly revealed the historical use of *Spirulina maxima* and *Spirulina platensis* species as human food.

*Spirulina* proteins contain a large quantity of phycobiliprotein (about 20% of the total protein content), including phycocyanins, known for their attractive blue color and tremendous health effects on the human organism, as demonstrated by a large volume of literature (Romay et al., 1998; González et al., 1999; Bhat et al., 2001; Madhava et al., 2003). *Spirulina* appears to be able to yield a high protein content, with up to 77% for *Arthrospira maxima* (Paoletti et al., 1980) (**Table 1**) on a dry weight basis.

*Aphanizomenon* sp. are filamentous free-floating solitary algae. In a few species, these algae are joined into fascicle-like, microscopic or macroscopic (up to 2 cm long) colonies with trichomes oriented in parallel. They are prokaryotic cyanobacteria of the *Nostocaceae* family. In comparison with *Arthrospira*, *Aphanizomenon flos-aquae* has only recently been used for human consumption. The exploitation of *A. flos-aquae* started in the early 1980s (Carmichael et al., 2000). This organism is capable of yielding a protein content on a dry matter basis of 62% (**Table 1**) (Becker, 2007). Some studies have revealed the presence of hepatotoxic microcystins in foods supplemented with *A. flos-aquae* at the level of 0.1–4.72  $\mu\text{g}\cdot\text{g}^{-1}$  (Saker et al., 2005), but their source remains unknown. These toxic substances may have resulted from exogenous contamination.

*Anabaena* sp. is among the filamentous microalgae species also known as cyanobacteria. The species has a high ability to fix nitrogen and to remove  $\text{CO}_2$  from polluted water (González López et al., 2010). Despite their relatively high protein content (43–56%) (Becker, 2007), these organisms represent one of the four genera of cyanobacteria biomass that have been shown to contain neurotoxins (*Anabaena* sp., *Aphanizomenon* sp., *Planktothrix agardhii* and *Microcystis* sp.) (Pawlik-Skoronska et al., 2013).

*Chlorella* sp. is a green microalga of the phylum chlorophyta. It has a spherical shape of about 2 to 10  $\mu\text{m}$  in diameter (Scheffler, 2007). Its protein content shows a wide range among the strains of the species, starting from as low as 6.87% for the species *Chlorella spaerckii* (Slocombe et al., 2013) to as high as 58% for *Chlorella vulgaris* (Becker, 2007) (**Table 1**). *Chlorella spaerckii* and *Chlorella ovalis* (10.87%) (Slocombe et al., 2013) appear to be poor sources of protein, and the mass production of these strains would therefore be better directed towards other uses, such as lipid extraction for biofuel synthesis. However, the proteins that are produced could be exploited as by-products after lipid extraction.

*Scenedesmus* sp. is also a eukaryotic microalga, which features among the most common freshwater genera. *Scenedesmus almeriensis* is a novel strain of *Scenedesmus* sp., which presents many advantages such as a high growth rate, and tolerance to both high temperatures and high copper concentrations. In addition to its high protein content, *Scenedesmus* sp. is also an important source of carotenoids, especially lutein, which is known for its protective effect against photochemical damage of the macular region of the eye (Landrum, 1997). The protein content of *Scenedesmus* sp. has been found to be in the range of 30–50%, depending on the cell disruption pre-treatment method applied (González López et al., 2010). A protein content of 41.8% for the strain *Scenedesmus almeriensis* was reported by Romero et al. (2012).

In his study, Becker (2007) reported a protein content for *Scenedesmus obliquus* of between 50 and 55%, while González López et al. (2010) reported 48%. As for most microalgae, the protein content of *Scenedesmus* has been shown to vary widely (30–55%), depending on the cell disruption pre-treatment method applied, the strain of the species and probably the nature of the growth medium, among other factors.

*Tetraselmis* sp. is a genus of green unicellular flagellated eukaryotic microalgae (usually 10  $\mu\text{m}$  long x 14  $\mu\text{m}$  wide). Most strains possess 8 flagella, while a few possess 16 flagella.

*Tetraselmis* is characterized by its high lipid content (22%) but the protein level is also relatively high (31–36%) compared to some high protein sources, such as soya bean (37%) and milk (26%) (Brown, 1991; Becker, 1994; Schwenzfeier et al., 2011).

*Porphyridium* sp. is a eukaryotic red microalgal species belonging to the Porphyridiaceae family and the Porphyridiophyceae class. It has a high carbohydrate content, and is especially known for the beneficial health effects of its sulphated polysaccharides (Patel et al., 2013). The protein content of *Porphyridium cruentum* has been shown to vary from approximately 30% to 35%, depending on the cell disruption pre-treatment applied (González López et al., 2010). A recent study by Sean et al. (2015), on the chemical composition of some microalgae species, revealed the crude protein content of the species *Porphyridium aerugineum* to be 31.6% on a dry weight basis.

The halotolerant biflagellated alga *Dunaliella* sp. is known for its orange-red pigment  $\beta$ -carotene, a precursor of vitamin A. *Dunaliella* proteins are reported to play a role in fighting against the transient intracellular salt fluctuations during hyperosmotic or hypoosmotic shocks (Chen et al., 2015). Results from a study by Tavallaie et al. (2015) on the protein production of *Dunaliella salina* showed that protein production and pigmentation correlated well with the growth rate of the strain. *Dunaliella* sp. is one of the few microalgae that are produced commercially on a relatively large scale (Ben-Amotz, 2004). Becker (2007) reported a protein content of 57% for the species *D. salina* on a dry weight basis. Lower levels of protein content have been reported for the species *Dunaliella primolecta* (12.26%) (Slocombe et al., 2013) and *Dunaliella tertiolecta* (11.4%) (Barbarino et al., 2005).

#### 4. NUTRITIONAL AND FUNCTIONAL PROPERTIES OF MICROALGAE PROTEIN

Parameters measured for protein quality include the protein efficiency ratio (PER) expressed as the mass gain per unit of consumed protein, tested on animals in short-term trials. Other parameters that have been measured for the nutritional quality of a protein are the determination of its biological value (BV), giving the proportion of absorbed protein, the protein digestibility coefficient (DC), and the net protein utilization (NPU) equivalent to a calculation of (BV) X (DC) (Becker, 2007) (Table 2). The method chosen for drying the microalgae biomass seems to have an impact on these parameters. *Chlorella* sp. DD, *Spirulina* sp. SD, and *Scenedesmus obliquus* DD have shown the highest values among the tested microalgae biomass, values that are not far from those of the referenced sources casein and egg. To some extent, these microalgae may be able to readily replace the conventional protein sources in a diet.

One of the quality criteria for a protein is its amino acid composition, specifically the essential amino acids.

**Table 2.** Comparative data on biological value (BV), digestibility coefficient (DC), net protein utilization (NPU), and protein efficiency ratio (PER), of differently processed algae (Becker, 2007 based on Becker, 2004; Richmond, 2004) — *Données comparatives sur la valeur biologique (VB), le coefficient d'utilisation digestive (CUD), l'utilisation nette des protéines (UNP) et le rapport d'efficacité protéique (REP) des algues transformées de différentes manières (Becker, 2007 basé sur Becker, 2004; Richmond, 2004).*

	Processing	BV	DC	NPU	PER
Casein		87.8	95.1	83.4	2.50
Egg		94.7	94.2	89.1	—
Alga					
<i>Scenedesmus obliquus</i>	DD	75.0	88.0	67.3	1.99
<i>Scenedesmus obliquus</i>	DS	72.1	72.5	52.0	1.14
<i>Scenedesmus obliquus</i>	Cooked -SD	71.9	77.1	55.5	1.20
<i>Chlorella</i> sp.	AD	52.9	59.4	31.4	0.84
<i>Chlorella</i> sp.	DD	76.0	88.0	68.0	2.00
<i>Spirulina</i> sp.	SD	77.6	83.9	65.0	1.78
<i>Spirulina</i> sp.	DD	68.0	75.5	52.7	2.10

AD: air dried — séché à l'air; DD: drum dried — séché sur tambour; SD: sun dried — séché sous le soleil.

There is evidence in the literature showing that, in this regard, algae protein can readily compare with proteins derived from conventional sources. Values for amino acid content (Table 3), reported by Kent et al. (2015), compare well with those presented by Christaki et al. (2011). The slight differences shown may be due to the differences between the tested microalgae species or the origin of the analyzed biomass. All essential amino acid levels reported for the various microalgae species also compare well to those of the conventional sources of protein. The essential amino acid cysteine appears to have been the same limiting amino acid for egg and for most of the analyzed microalgae species. Microalgae as a protein source can readily substitute for egg or other animal protein. Their amino acid profile is far superior to that of soybean and to that of other plant sources when considering the essential amino acid content.

Some microalgae proteins exhibit good functional properties and are readily integrated into food formulations for health management. Phycobiliproteins found in cyanobacteria, such as *Spirulina* sp., *Anabaena* sp., and *Oscillatoria* sp., are colored proteins, recognized for their large number of health applications, and they have been intensively

**Table 3.** Amino acid profile of different microalgae as compared with conventional protein sources (g·100 g<sup>-1</sup> protein) — Profil en acides aminés de différentes microalgues comparé à celui des sources conventionnelles de protéines (g·100 g<sup>-1</sup> de protéine).

Source	Ile*	Leu*	Val*	Lys*	Phe	Tyr	Met*	Cys*	Trp*	Ala	Arg	Asp	Glu	Gly	His*	Pro	Ser	Reference
Egg	6.6	8.8	7.2	5.3	5.8	4.2	3.2	2.3	1.7	5.0	6.2	11.0	12.6	4.2	2.4	4.2	6.9	Christaki et al., 2011
Soybean	5.3	7.7	5.3	6.4	5.0	3.7	1.3	1.9	1.4	4.0	7.4	1.3	19.0	4.5	2.6	5.3	5.8	Christaki et al., 2011
<i>Aphanizomenon</i> sp.	2.9	5.2	3.2	3.5	2.5	—	0.7	0.2	0.7	3.3	3.8	4.7	7.8	2.9	0.9	2.9	2.9	Christaki et al., 2011
<i>Arthrospira maxima</i>	6.0	8.0	6.5	4.6	4.9	3.9	1.4	0.4	1.4	4.6	6.8	8.6	12.6	4.8	1.8	3.9	4.2	Christaki et al., 2011
<i>Chlorella</i>	4.4	9.2	6.1	8.9	5.5	4.2	2.2	0.4	—	4.7	8.3	7.1	9.4	5.4	2.4	4.8	4.0	Kent et al., 2015
<i>Dunaliella</i> sp.	4.5	9.3	6.0	6.2	6.0	4.0	2.5	4.0	—	5.0	7.8	6.6	10.5	5.7	2.5	4.9	4.4	Kent et al., 2015
<i>Dunaliella bardawil</i>	4.2	11.0	5.8	7.0	5.8	3.7	2.3	1.2	0.7	5.4	7.3	10.4	12.7	5.5	1.8	3.3	4.6	Christaki et al., 2011
<i>Scenedesmus</i> sp.	4.7	9.4	6.0	6.8	5.5	4.0	2.4	0.1	—	4.9	6.8	6.0	9.2	5.2	2.6	8.3	4.2	Kent et al., 2015
<i>Scenedesmus obliquus</i>	3.6	7.3	6.0	5.6	4.8	3.2	1.5	0.6	0.3	5.1	9.0	7.1	8.4	7.1	2.1	3.9	3.8	Christaki et al., 2011
<i>Spirulina</i>	5.8	9.0	6.4	5.1	4.8	4.8	2.9	0.3	—	5.1	7.4	7.6	10.2	4.6	2.0	3.3	4.8	Kent et al., 2015
<i>Spirulina platensis</i>	6.7	9.8	7.1	4.8	5.3	5.3	2.5	0.9	0.3	6.2	9.5	7.3	11.8	5.7	2.2	4.2	5.1	Christaki et al., 2011

\*: essential amino acid — acide aminé essentiel.

investigated for this purpose. These proteins include allophycocyanin, phycocyanin, and phycoerythrin.

A molecule of phycocyanin is made up of two subunits linked together: a protein unit and a bilin chromophore unit (**Figure 1**). The chemical structure of the bilin chromophores in phycocyanin is close to that of bilirubin, a heme breakdown product. Bilirubin acts as an antioxidant and cytoprotector for tissues such as myocardium and nervous tissue, by scavenging oxygen free radicals (Temme et al., 2001).

## 5. PHYSICOCHEMICAL AND TECHNOFUNCTIONAL PROPERTIES OF ALGAL PROTEINS

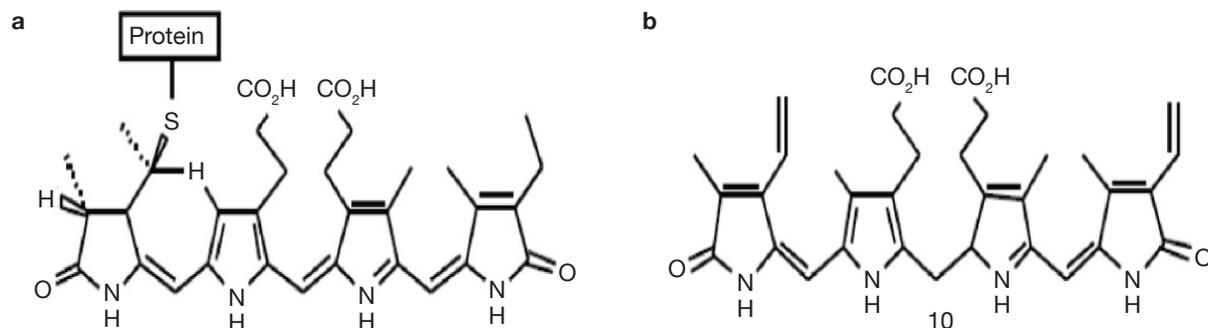
The term “technofunctional” has been suggested to describe the properties of proteins and other nutrients that, in addition to their nutritional function, are capable of playing other roles in food formulation. The technofunctional properties of proteins include all the physicochemical properties that would affect their technological applications during processing, handling and consumer use. These properties are strongly involved in enhancing the organoleptic characteristics of processed food, including texture, viscosity, palatability, mouth feel, etc. The technofunctional properties of food proteins involve two molecular aspects (Damodaran, 1989), *i.e.* protein-surface related properties (solubility, wettability, dispersibility, foaming, emulsification, flavor and fat binding) and hydrodynamic properties (viscosity, thickening, gelation, texturization).

Although proteins are the main focus of interest with respect to substances obtained from microalgae, their characterization remains incomplete. Only a few scientific publications are available on the technofunctional properties of algal protein, mainly relating to the soluble protein fractions of *Spirulina* sp. and *Tetraselmis* sp. Examples of such properties are highlighted below.

Chronakis et al. (2000) found that the soluble protein fraction of *Spirulina platensis* had remarkable properties in reducing the surface tension.

More recently, Mahajan et al. (2010) compared native and chemically modified *Spirulina* proteins for their functional properties. Results showed that *Spirulina* protein solubility in water decreased considerably when treated with succinic anhydride, acetic anhydride and formaldehyde. Foam stability was found to be higher with methylation and acetylation. Maximum viscosity was obtained with the succinic anhydride modified protein fraction followed by acetylation.

The emulsifying and foaming properties of *Tetraselmis* soluble protein isolate were investigated



**Figure 1.** Chemical structure of phycocyanobilin chromophore (open-chain tetrapyrrol) (a) and bilirubin (b) — *Structure chimique du chromophore de phycocyanobiline (chaîne ouverte tetrapyrrole) (a) et de bilirubine (b)* (Romay et al., 2003).

by Schwenzfeier et al. (2011; 2013). This algae isolate appeared to be a mixture of mainly proteins and polysaccharides. Since *Tetraselmis* allows the formation of stable emulsions at low protein concentrations, it can be considered as an efficient natural alternative to existing protein-polysaccharide complexes such as gum arabic. Moreover, the overall foam stability of the algae protein-rich fraction was found to be superior to that of whey protein isolate and egg white albumin in the pH range from 5 to 7.

## 6. CONCLUSIONS

Microalgae are quantitatively and qualitatively good protein sources. Their potential use in human nutrition has not yet been fully realized, and is currently generally limited to functional food ingredients. Some species, such as *Chlorella* sp., *Aphanomezinon* sp., *Nostoc* sp., and *Spirulina* sp., have an historical use in human nutrition, but they are still more or less confined to their natural areas of production, and their industrial production remains limited. Mass production of microalgae needs to be encouraged by public authorities in order to help overcome food shortages for the growing global population. However, the complete toxicological, microbiological and biochemical status of algal products would need to be determined before their full integration as basic ingredients into processed foods, whether for functional or solely nutritional purposes.

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