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Green Microalgae as a Food Source – Growth Kinetics and Biochemical Composition

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ABSTRACT

Microalgae are considered as a renewable natural resource that presents important potentialities to be valorized in several fields. This valorization must necessarily start with a thorough study of the biochemical composition of each species of algae. The objective of this study is to study the evolution of the biochemical composition according to the different stages of growth of three biomasses of microalgae (*Fragilaria sp, Scenedesmus protuberans*, *Polytoma Papilatum*) collected from Moroccan aquatic environments. *Polytoma Papilatum* and *Scenedesmus protuberans* show high protein content of 89.23±2.58%, 90.4±1.45% respectively in addition to low lipid 2.4±0.23, 1.63±0.2% and carbohydrate 8.08±1.25, 8.19±1.07 respectively. On the other hand, *Fragilaria sp* has high value of carbohydrate 65.73±3.25% as well as low in protein and lipid contents with values of 33.16±1.76, 1.28±0.29 respectively. The monitoring of the growth kinetics allows differentiating three phases on the growth curve: latent phase, exponential growth phase, and stationary phase. Regarding the biochemical composition, the highest content of proteins, carbohydrates and lipids in relation to the harvested biomass reach its maximum at the stationary phase.

Keywords: algae, biomass**,** protein, lipid, carbohydrate.

INTRODUCTION

Microalgae, lower plants endowed with autotrophy by photosynthesis, actively participate in the production of atmospheric oxygen (John, 1994) and constitute the phytoplankton at the base of the food chain of the aquatic environment. Microalgae constitute a breeding ground for new scientific and economic applications. Their most marked biochemical characteristics concern lipids, proteins, and polysaccharides.

These molecules are at the basis of many developments in fields as varied as health, human or animal food, (Muller-Feuga, 1997; Niccolai et al., 2019) or the environment with, for example, the production of hydrocarbons. Microalgae are a source of a wide range of natural products, including carbohydrates, proteins, lipids, they have good commercial prospects: in energetics, food, pharmaceuticals and other high-value products (Anjos et al., 2013; Benavente-Valdés et al., 2016; D'Alessandro and Antoniosi Filho, 2016). Determination of biochemical composition and digestibility is the first requirement to evaluate the potential of novel food sources (Niccolai et al., 2019). In this work, three microalgae biomasses belonging to the species harvested from Moroccan aquatic environments were tested in vitro for their biochemical composition to evaluate their potential application as food sources.

MATERIALS AND METHODS

Sampling

The selected species were sampled in November 2022 using a 45 µm mesh at the Idriss 1 dam site (34°7'31.989"N; 4°39'50.032 "W) in the Fez region (Northern Morocco). They were isolated after 150 trials and identified by molecular analysis (Table 1). Isolation of the cells allows obtaining monospecific cultures. It is recommended to use cultures in exponential growth phase, i.e. composed of young cells with a good multiplication potential.

Culture medium and microalgae growth

All experiments were conducted in Bold medium (Kord et al., 2012) at an initial pH of 6.5±0.2, 27°C, and under continuous illumination of 165 µmol m 2s-1 (24 h per day). This culture medium was chosen because it contains the necessary components for growth conditions. The employed culture was performed in a culture chamber photoperiod 14/10 whose luminosity is 6500 Lux, the temperature was 30°C during the day and 27°C at night, the cultures were launched in a photobioreactor of 29.5cm height and 11cm diameter, containing 2 liters of culture medium.

Determination of algal biomass

Three 100 ml samples were taken from each photobioreactor for dry biomass determination. Then, centrifugation was performed at 10,000 g and 4C for 10 minutes to separate the biomass from the culture medium. Afterwards, the supernatant was removed from the tubes and the wet biomass pellet was washed with deionized water to remove salts. Then another centrifugation was performed to separate the biomass from the deionized water. The resulting wet biomass was placed in Petri dishes with an average weight of 4.28 g and placed in an air circulation oven at 60°C. A series of weight measurements were performed each time using a 0.0001 precision balance to obtain a constant mass. Biomass was calculated using the following formula according to (Dos Santos et al., 2013):

$$X = \frac{mf}{\text{Vol}} \quad (1)$$

where: X – concentration of the biomass in the photobioreactor in mg/L; mf – final dry weight in mg; Vol – volume of the centrifuged sample, in L.

Protein, carbohydrate and fat composition

The samples for protein, carbohydrate, and lipid determination were filtered on Whatman GF/C filters of 0.45 µm porosity, previously calcined at 550°C for 5 hours (for decontamination as well as demineralization). A single filter for each species is intended for protein and carbohydrate determination. Total protein content was determined by using the Folin phenol method (Lowry et al., 1951). Total carbohydrate content was determined by the phenol-sulfuric acid method using glucose as a standard (Dubois et al., 1956). We used the modified method (Bligh and Dyer, 1959) was employed, since it meets the solvent system criterion for lipid extraction.

RESULTS AND DISCUSSION

The growth kinetics of microalgae was evaluated in batch cultures. Three phases were differentiated on the growth curve: latency phase, from the beginning of the culture to the 9th day; growth phase, from the 9th to the 18th day and the stationary phase, from the 18th to the 24th day (end of the culture). The microalgae cultures reached a biomass concentration of 44.17 mg/L, 3.46 mg/L and 1.16 mg/L over a period of 21 days for Fragilaria sp, Polytoma Papilatum and Scenedesmus protuberans respectively, showing a typical growth curve for microalgae batch cultures (Fig. 1).

Fragilaria sp

Globally the variations of protein concentrations follow those of the biomass, these concentrations drop on the 21st day when the concentration reaches 25.4 µg/ml. The protein content oscillates between 14.0µg/ml and 14.99µg/ml recorded on the first and last day of the culture and 105.25 µg/ml recorded on the 18th day, end of the exponential phase. Relative to the biomass, the protein content is 0.05% recorded on day 21, stationary phase (Figure 2). The carbohydrate concentration varies between 1.81 µg/ml recorded on the 6th day of the culture, end of the latent phase and 31.14 µg/ml on the 21st day, the stationary phase. On the 24th day, a sharp decrease of carbohydrates which reach 4.8 µg/ml. The carbohydrate

Table 1. Species isolated from different sites

Species	Order	Family	Sampling site
Fragilaria sp.	Fragilariales	Fragilariophycidae	Idriss 1 Dam
Polytoma Papilatum	Chlamydomonadales	Chlamydomonadaceae	Idriss 1 Dam
Scenedesmums quadricauda	Chlorococcales	Scenedesmaceae	Idriss 1 Dam

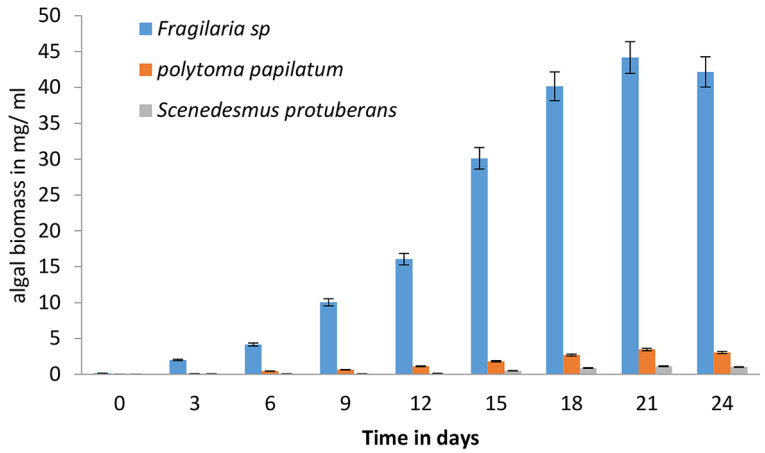

Figure 1. Variation in algal biomass of *Fragilaria sp., Polytoma Papilatum* and *Scenedesmus protuberans* in time function

content in *Fragilaria sp* is higher than the protein content, it is 0.1% of the biomass recorded on day 21 stationary phase (Figure 2). The concentration of lipids in *Fragilaria sp.* varies between 0.62 µg/ml on the last day of culture stationary phase, 6.61 µg/ml on day 15 middle of the exponential phase. In contrast to carbohydrates, lipids show an increase during the latent phase and the middle of the exponential phase followed by a strong decrease for both compounds, lipids and carbohydrates, except that this decrease was at the end of the exponential phase for carbohydrates. The lipid content equals 0.002% of the biomass recorded on day 21 of the stationary phase (Figure 2).

Polytoma Papilatum

The protein variation curve shows a general pattern similar to that of the biomass during the culture, it varies between 17.93 µg/ml on the first day of the culture and 436.01 µg/ml on the last day. An increase of the concentration on the

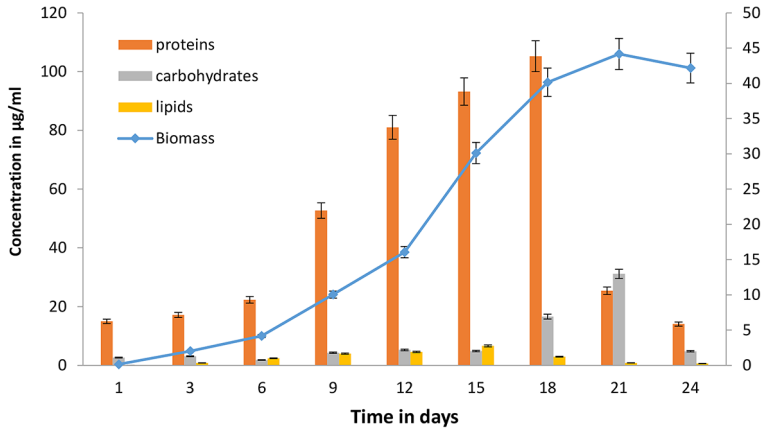

Figure 2. Temporal variations in protein, carbohydrate, lipid and biomass concentrations of *Fragilaria sp* in culture; axis 1 represents the concentrations of proteins, carbohydrates and lipids and axis 2 represents the biomass content

3rd day at the end of the latency phase with a value of 89.79 µg/ml. Relative to the biomass, the protein content is 10.04% recorded on day 21 at the end of the exponential phase (Figure 3). The carbohydrate content in *Polytoma Papilatum* varies between 2.9 µg/ml recorded on the 3rd day of culture which coincides with the end of the latent phase and 34.62 µg/ml recorded on the 21st day end of the exponential phase. The value that represents the carbohydrates in relation to the biomass in the 21st day end of the exponential phase is 1% (Figure 3). The lipid curve has almost the same pattern as the carbohydrate curve. The concentration of lipids in *Polytoma Papilatum* is 2.03 µg/ml recorded on the first day of the culture, i.e. the latent phase and 12.27 µg/ml recorded at the end of the decay phase which coincides with day 24. Regarding the percentage of lipids to biomass is 0.26 on day 21 the end of the exponential phase (Figure 3).

Scenedesmus protuberans

The highest value of protein in *Scenedesmus protuberans* 464.34 µg/ml is recorded on the 18th day, exponential phase and the low value 13.54 µg/ml is recorded on the 6th day middle of the latent phase. The protein content in relation to the biomass is 4.47% (Figure 4). The carbohydrates in *Scenedesmus protuberans* start from 0,4µg/ml on the first day of the culture to 5,16 µg/ml on the 18th day, which coincides with the exponential phase. A considerable increase on day 9 with a value of 2.9 µg/ml. Towards the end of the exponential phase the concentration of carbohydrates decreases with the biomass curve to reach 3.17 µg/ml. Relative to the biomass, the protein content recorded on day 21 at the end of the exponential phase is 0.04% (Figure 4). Both curves (lipids and biomass) have the same shape from the first day of the culture until the 18th day towards the

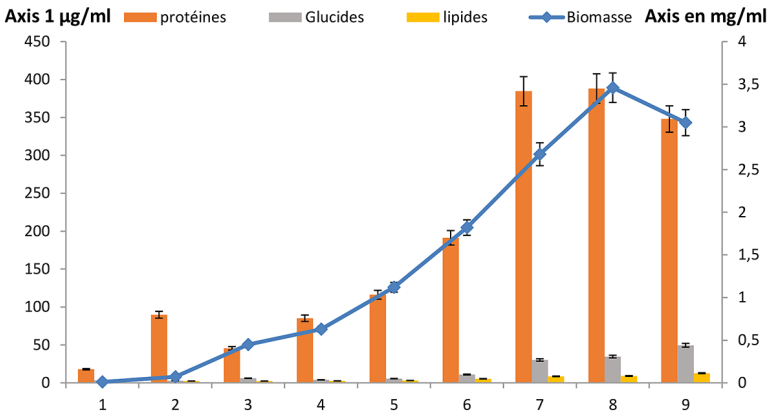

Figure 3. Temporal variations in protein, carbohydrate, lipid and biomass concentrations of *Polytoma Papilatum* in culture; axis 1 represents the concentrations of proteins, carbohydrates and lipids and axis 2 represents the biomass content

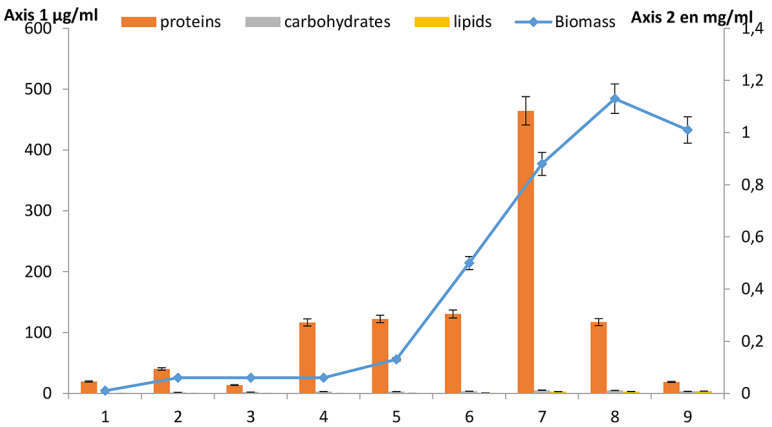

Figure 4. Temporal variations in protein, carbohydrate, lipid and biomass concentrations of *Scenedesmus protuberans* in culture

end of the exponential phase after this day the lipid content shows a striking drop to reach 0.52 µg/ml at the end of the culture. The minimum lipid value in *Scenedesmus protuberans* is 0.072 µg/ml recorded on day 13 which is consistent with the beginning of the exponential phase. The percentage of lipids in relation to the biomass recorded the lowest value compared with the other components, 0.08% on day 21 (Figure 4).

Several species of microalgae can be exploited in various fields of food or industrial interest. However, the valorization of algal biomass requires a perfect knowledge of the biochemical composition of these species and of the variations, which can be induced under the influence of environmental factors. In this perspective, the biochemical characterization of the main cellular constituents (proteins, carbohydrates, lipids) of three species of Moroccan algae was undertaken. The analyses carried out reveal that protein, carbohydrate and lipid contents vary from one species to another, which may be due in addition to interspecific variations (Morris, 1981) to the sites of origin. They even vary according to the growth stages, which is shown by this study and which corroborates with those demonstrated by (Belkoura et al., 1997; Tahiri et al., 2000). In the same sense (Hu, 2004) indicates that the content of the main biochemical components of algal cells varies depending on the microalgae studied and the culture conditions, growth phase and physiological state. The analytical technique adopted to determine the different components may lead to differences in the final results. Also (Hu et al., 2008; Khozin-Goldberg and Cohen, 2011; Msanne et al., 2012) reported that temperature factors, irradiation and nutrient availability affect the biochemical compositions of many algal species.

The protein content of the examined microalgal biomasses was similar to the value found in the literature (Idrissi 2016; Yoo et al., 2010). Microalgae, especially cyanobacteria, generally have higher protein content than protein crops such as legumes (FAO/WHO, 1991, Pimentel, 2009). It is possible to enrich the diet with about 6% of daily protein requirements (FAO/WHO, 2007). Lee et al. (2015) referred to the accumulation of nutrients such as starch and lipids during the stationary phase as a survival strategy for microalgae in the face of nutrient limitations. The decrease in protein concentration at the end of the stationary phase could be associated with the increase in carbohydrates in the same phase. The synthesis of proteins, carbohydrates and lipids in microalgae is influenced by several factors that vary their content. Among these factors are temperature, nitrogen deficiency and its nature, pH, photoperiod, light intensity, nutrient availability, CO_2 concentration, as well as osmotic pressure of the environment (Yoo et al., 2010; Cassidy K.O. 2011; Aurore V. 2013; George et al., 2014; Pancha et al., 2014). This multitude of parameters accounts for the complexity of the mechanisms of regulation of the metabolism of the biochemical components of these organisms. The work of Sabatie et al (1986) and Dermoun (1987) showed that the polysaccharides produced by algae undergo structural modifications according to the culture parameters mentioned above. Therefore, any attempt to valorize these constituents should take into account this aspect through a precise analysis of the nature and structure of the synthesized carbohydrate molecules.

While comparing the protein, carbohydrate, lipid content for each species, it was found that protein occupies the important part followed by carbohydrate and lipid for *Polytoma Papilatum*, *Scenedesmus protuberans*, except for *Fragilaria sp* where carbohydrate ranked first followed by protein and lipid (Table 2). Thus, the graphical representations show that protein contents show a similar growth to that of biomass and maximum values are recorded at the end of the growth phase, followed by a strong decrease at the end of the stationary phase.

Table 2. The percentage of proteins, carbohydrates, lipids of the studied microalgae species at the end of the exponential phase

Specification	*Fragilaria* sp.	*Polytoma Papilatum*	*Scenedesmus protuberans*	F-value	p-value	LSD 0.05
Proteins	33.16 ±1.76^{a}	89.23±2.58^{b}	90.4±1.45^{b}	813.64	< 0.0001 (Significant)	3.97
Carbohydrates	65.73±3.25^{a}	8.19±1.07^{b}	8.08±1.25^{b}	747.92	< 0.0001 (Significant)	6.82
Lipids	1.28±0.29^{a}	2.4±0.23^{b}	1.63±0.2^{c}	15.43	0.004 (Significant)	0.62

CONCLUSIONS

This research aimed to determine how protein, carbohydrate and lipid content vary with growth kinetics and biomass quantity. In the present study, the possibility of valorizing the microalgae as a food source was confirmed. The value of the prospects of these microalgae for various applications was presented in terms of its biochemical components. *Polytoma Papilatum* and *Scenedesmus protuberans* show high protein and low lipid and carbohydrate content. On the other hand, *Fragilaria sp* presents a high value in carbohydrates and low in proteins and lipids, the maximum values of these biomolecules was obtained in the stationary phase. These works provide encouraging results for the industrial development of cultures of the three species of microalgae with an excellent yield of biomass and biomolecules during the stationary phase.

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