

**Proximate analysis, lipid profile,  
microbiological and pigment characterization of  
*Chlorella* dry powder produced in a 20 L  
agitated photobioreactor**

**ABSTRACT**

**Aims:** The aim of this work was to investigate the massive production of *Chlorella* with food purposes. The second objective was the characterization of the dried product, in terms of proximal, microbiological, pigment and specific lipids analysis. **Place and Duration of Study:** This work was carried out mostly in the bioprocess laboratory, UPIBI-Instituto Politecnico Nacional in 2016. **Methodology:** The *Chlorella* Powder was cultivated in a 20 L agitated tank. *Chlorella* was separated from the media, dried and characterized in terms of proximal analysis, lipid profile, microbiological and pigments. Results were compared with a commercial product. **Results:** It was feasible the production of *Chlorella* in a 20 L agitated reactor in outdoor conditions. The calculated value of  $\mu$  was  $0.093 \text{ day}^{-1}$ . The *Chlorella* product presented similar characteristics to a commercial product maybe except in the protein content (the commercial *Chlorella* had a protein contain up to 40% and the home-made product, 21%). The microbiological characterization indicated that both home-made and commercial products are able to be used as human food or supplement. The analysis of pigments by TLC showed the presence of zeaxanthine,  $\beta$ -carotene, chlorophyll a, pheophorbide and pheophytine. The lipids of the home-made product were composed basically of  $\alpha$ -linoleic acid (23.4% in molar basis) and palmitic acid (17.5%), followed by linoleic (13.5%) and stearic acids (12%), while the commercial product lipids were composed mainly by  $\alpha$ -linoleic (26.9%) and palmitoleic acids (19.1%), followed by palmitic acid (18.9%). **Conclusion:** It was feasible the production of *Chlorella* in a 20 L agitated reactor in outdoor conditions. The home-made product is very similar to the commercial one. It can be used as nutraceutic, providing with proteins, minerals, antioxidant and healthy lipids to the consumer.

**Keywords:** agitated photobioreactor, *Chlorella*, food, lipids, pigments, proteins.

**1. INTRODUCTION**

Microalgae such as *Chlorella* can be massively produced using different systems including airlift loop bioreactors ALB (Ying et al 2013), bubble-column photobioreactors BCP (Loures et al, 2015) or agitated photobioreactor AP (Kumar et al, 2016) in both batch and chemiostate operation modes. Employed growth medium is in general a defined salts medium, but the use of different wastewaters from different sources has been also reported (Zhang and Hong, 2014).

There are different applications for *Chlorella* production. One of them is the use of the dry microalgae in order to extract the lipids produced and use them for biodiesel production (Mostafa et al, 2014; Zhang and Hong, 2014; Loures et al, 2015; Kumar et al, 2016). The

other biomass application is as human or animal supplement. (Seyfabadi et al, 2011; Bishop and Zubeck, 2012; Kent et al, 2015).

Nutraceuticals are food or part of food that not only supplement the diet, but also facilitate the prevention or treatment of a disease and/or disorder (Bishop and Zubeck, 2012). The current estimated global market size for nutraceutical products is 30-60 billion dollars. Microalgae are a diverse group of autotrophic organisms that have the ability to grow rapidly, efficiently use light energy, fix atmospheric CO<sub>2</sub>, and produce more biomass per acre than plants. Microalgae that are currently employed as nutraceuticals are *Chlorella*, *Dunandiella*, *Haematococcus*, *Aphanizomenon* and *Spirulina*. This is due to their vitamins, K, Na, I, Se, Fe, Mn, Cu, P, Na, N, Mg, Co, Mo, S and Ca contents. These algae are also producers of essential aminoacids and specific lipids, such as omega 6 and decosahexanoic acid. (Bishop and Zubeck, 2012).

Regarding the use of *Chlorella* as nutraceutic, it has been reported that this microalgae posses diverse antitumor, antioxidant, anti-inflammatory and antimicrobial activities, *Chlorella* is able to decrease blood pressure, lower cholesterol levels, accelerate wound healing and enhance the immune system (Bishop and Zubeck, 2012). The United States, Japan, China, Taiwan and Indonesia produce over 2,500 tons of dried *Chlorella* each year (Gupta and Mukerji, 2001). It has been published that *Chlorella* dry powders contain about 40, 16, 25 and 6% of proteins, lipids, carbohydrates and ashes, respectively. Besides, *Chlorella* contains chlorophylls, carotenoids, astaxantine and  $\beta$ -carotene (Kent et al, 2015). Finally, Bishop and Zubeck (2012) found vitamins B<sub>1</sub>, B<sub>3</sub>, B<sub>5</sub>, B<sub>6</sub>, B<sub>9</sub> and B<sub>12</sub>, in *Chlorella* dry product besides chlorophyll,  $\beta$ -carotene and Mg.

The aim of this work was to show how feasible is the massive production (20L) of microalgae with food purposes, using a strain of *Chlorella*. The second objective is to determine some characteristics of the dried product, such as proximal analysis, microbiological characteristics pigment and lipids analysis.

## 2. MATERIAL AND METHODS

### 2.1 *Chlorella* strain

The employed *Chlorella* sp. strain was isolated from a consortium composed by *Chlorella vulgaris*, *Scenedesmus* sp. and some diatomeas employed in previous works (Torres et al, 2016).

### 2.2 Cultivation in the 20 L fotobioreactor

The cultivation was performed in a 20 L agitated fotobioreactor, under ambient conditions for 14 days in the BBM medium. Cultures were aerated 12 hours a day by a perforated ring placed at the base of the bioreactor. Biomass development was followed by reading optical density. Previously a calibration curve DO vs dry weight was prepared. The variables were measured throughout the process once a day (about 10 am) was biomass; pH, conductivity, outside temperature, and the photon irradiance  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  were measured. Harvest was carried out by sedimentation. The supernatant was repeatedly retired from the reactor until a small amount of sludge was obtained. Total amount of microalgae was centrifuged (HERMLE Z306 equipment) at 6,000 rpm during 30 min. The material thus produced was dried at environment conditions, and it was ground and stored for later proximate analysis. Commercial *Chlorella* product (Vidanat, Mexico) was characterized with comparison purposes.

### 2.3 Proximate composition

The proximate composition of products was determined according to the AOAC (1997) International methods, namely protein (954.01); fat (920.39); ash (923.03); crude fiber (962.09); humidity (925.09); and total carbohydrates calculated by differences. Equations employed were the following (in %):

$$\text{Protein (dry basis)} = \frac{\text{N HCl} \times \text{Corrected acid volume g of sample} \times 14 \text{ g N mol/ g of dried sample} \times 100}{\text{sample}} \times 6.25 \quad (1)$$

$$\text{Fat (dry basis)} = \frac{\text{g of fat in sample}}{\text{g of dried sample}} \times 100 \quad (2)$$

$$\text{Ash (dry basis)} = \frac{\text{wt after ashing} - \text{tare wt of crucible}}{\text{original sample wt} \times \text{dry matter coefficient}} \times 100 \quad (3)$$

$$\text{Crude fiber (dry basis)} = \frac{\text{g of crude fiber}}{\text{g of dried sample}} \times 100 \quad (4)$$

$$\text{Moisture (wt/wt)} = \frac{\text{wt H}_2\text{O in sample}}{\text{wt of wet sample}} \times 100 \quad (5)$$

$$\text{Carbohydrates} = 100 - (\text{P} + \text{F} + \text{A} + \text{CF} + \text{M}) \quad (6)$$

### 2.4 Microbiological characterization

The two dried products were analyzed in terms of fungi, yeast, total and faecal coliforms, *Salmonella* and *Shigella* contents, in accord to a Mexican Norm (SSA,1994).

### 2.5 Pigments analysis

The pigments present in the dry product (home-made and commercial products) were analyzed as follows: 0.5 g of product was mixed with acetone for analysis and vortexed. Then the mud was put in a ceramic mortar and milled successively, adding as much as acetone as necessary (about 5 times). From this mud, take only 0.1 g and suspend in 1 mL of acetone in an eppendorf tube. Close and maintain from this point on in ice and covered with aluminum foil to prevent light damage. The eppendorf tube was centrifuged at 16,000 rpm during 10 min and maintained at low temperature (4°C). Take out the pellet repeat centrifugation step. Reserve the acetone containing the pigments. Prepare about 100 mL of a mixture of petroleum ether (70%) and acetone (30%) in a large precipitation flask was the TLC can be collocated. The TLC layers employed were 5x20, 0.2 mm aluminum oxide N (Macherey-Nagel Co, Germany). The layer is charged in the bottom side with 10 mL of every pigment solution, very slowly and carefully trying to produce a small spot over the layer. The mixture of solvents is inside the precipitates flask and is in such amount than when the layer is put inside the flask, the pigment spots do not touch the solvent. The layer is collocated in an inclined way and the flask is covered with aluminum foil in order to prevent the solvent evaporation as much as possible. The solvent starts to run through the layer and the pigments start to separate. The process is allowed until the solvent is 1-2 mm before the end of the layer. The layer was taken out from the flasks and allowed to dry completely. Using a small spatula, every pigment from the layer is scratched and suspended in 1 mL of acetone (80%) and water (20%) in an eppendorf tube, and centrifuged. This process is repeated if still some aluminum oxide particles are evidently floating on the solvent. Every pigment solution is read in a UV/Vis spectrophotometer (Perkin-Elmer Lambda 25). The profiles of abundance vs. wave length are stored and compared against those profiles previously reported in Jeffrey et al (1997).

### 2.6 Lipids isolation and quantification

Lipids were extracted following the method of Bligh and Dyer (1959) and then, were saponified and methylated by the method of Slover and Lanza (1979) Fatty acid profiles were obtained using a FOCUS gas-chromatography instrument (Thermo Electron Corporation, Les Ulis, France) equipped with a flame-ionization detector.

### 3. RESULTS AND DISCUSSION

#### 3.1 Growth of *Chlorella* in the photobioreactor

Table 1 show the parameters measured during the growth of *Chlorella* in the 20 L photobioreactor. *Chlorella* strain started to grow immediately (no lag phase was observed). At day 14<sup>th</sup> reached the maximum biomass of about 1.4 g/L. This value is higher to that reported by Mostafa et al (2012) for *Chlorella vulgaris* in municipal wastewaters at flask level at pH= 8.11 (1.052 g/L). The calculated value of  $\mu$  is 0.093 day<sup>-1</sup>. This value is lower to that reported by Zhang and Hong (2014) for *Chlorella sp.* growing in different wastewaters in sterile conditions ( $\mu$ = 0.39-0.47 days<sup>-1</sup>) but similar to the growing rate, when *Chlorella* grew in non-sterile conditions ( $\mu$ = 0.07-0.19 days<sup>-1</sup>). On the other hand, Chiu et al (2009) grew a strain of *Chlorella* in three different air-lift photobioreactors, reaching final biomass concentrations of 2.3-3.4 g/L and  $\mu$  values of 0.15-0.25 days<sup>-1</sup>, when *Chlorella* grew in artificial seawater for 11 days.

**Table 1.** Parameters measured at 10 am during the 14 days of *Chlorella* culture.

Day	Absorbancy (750nm)	Conductivity (ms)	pH	Irradiation ( $\mu\text{mol}/\text{m}^2\text{s}$ )	Temperature °C	Sky
1	0.066	26.3	9.77	1613	20	Clear
2	0.101	25.3	9.89	1400	22	
3	0.139	26.0	9.93	1782	21	
4	0.168	25.2	9.91	1883	20	
5	0.235	25.6	10.12	1710	21	
6	0.368	24.5	10.14	1600	22	
7	0.458	25.3	10.19	1660	20	
8	0.569	25.5	10.22	1500	21	
9	0.629	26.4	10.26	1340	22	
10	0.701	25.3	10.31	1450	20	
11	0.856	24.9	10.38	1420	21	
12	0.96	26.6	10.40	547	19	Cloudy
13	1.152	25.6	10.42	1250	20	Clear
14	1.256	25.4	10.45	780	19	Cloudy

As showed in the mentioned table, conductivity (as a measure of salinity) did not change very markedly during the process. pH values started in 9.8 and tend to increase up to the final of the culture, reaching a final value of 10.45. The daily irradiance received by the bioreactor was characterized as the rest of the parameters once a day (10 am). Irradiances fluctuated between 547 and 1,880  $\mu\text{mol photons}/\text{m}^2\text{s}$ . Days twelve and fourteen were very cloudy and irradiation was very low (547 and 780  $\mu\text{mol}/\text{m}^2\text{s}$  at 10 am, respectively). The rest of the days, sky was very clear and irradiation was higher than 1,250  $\mu\text{mol}/\text{m}^2\text{s}$ .

At the end of the process, the amount of biomass in the reactor was 1.4 g/L, meaning a biomass productivity  $P_X$  of 100 mg/L.day. Regarding the lipids, the final concentration was of 19 mg/L, giving a lipid productivity  $P_L$  of 1,35 mg/L.day. Regarding these values, Loures et al (2015) reported the growth of *Chlorella minutissima* in bubble column photobioreactors of 50L, using the Guillard f/2 medium. They reached final biomass values of 280-320 mg/L and biomass productivities between 40 and 46 mg/L.day (the half  $P_X$  that the one reached in this

work). Regarding the lipids, they reached final dry product concentrations between 31.5 and 34.3%, giving lipid productivities between 1,37 and 1.45 mg/L.day, very similar values to those obtained in this work.

Zhang and Hong (2014) reported that *Chlorella*, growing in different wastewaters reached biomass productivities  $P_X$  between 100 and 460 mg/L.day, while lipids showed productivities  $P_L$  between 0.8 and 4.25 mg/L.day.

### 3.2 Characteristics of the dry powder

The final product (around 25 g) was characterized in terms of the proximal analysis, microbiological parameters and pigments profile. The commercial product *Chlorella* Vidanat (Mexico) was also characterized with comparison purposes. Table 2 show the proximal analysis for the home-made and commercial *Chlorella* samples, as well as the results for a batch of dry *Spirulina* produced by our research group and published elsewhere (Torres et al, 2016).

It is noteworthy that the commercial and home-made *Chlorella* products are not so similar among themselves. While the carbohydrate content of commercial *Spirulina* is 6.52%, our home made product present 45.7%. Regarding the proteins level, the commercial product reached a concentration of about 38%, while the commercial sample showed a 21% content. Regarding lipids, the commercial product showed a concentration of 3.9% and our product 1.4%. Crude fiber showed contains were of 38.7 and 14.5 for the commercial and home-made product. Ashes showed values of 4.4 and 11.6% in the same order. Finally, humidity was quite similar with a content of 7.6% for the commercial *Chlorella* and 5.9 for our home-made product.

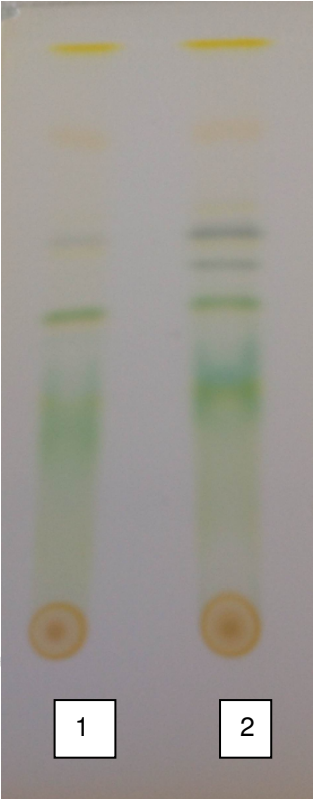
**Table 2.** Proximate analysis for the *Chlorella* dry products. Home-made *Spirulina* and commercial *Spirulina* are included with comparison purposes.

Sample	Carbohydrates (%)	Proteins (%)	Lipids (%)	Crude fiber (%)	Ashes (%)	Humidity (%)
Home-made <i>chlorella</i>	45.75	20.84	1.39	14.54	11.60	5.88
<i>Chlorella</i> vidanat	6.52	38.85	3.87	38.73	4.40	7.66
Home-made <i>Spirulina</i> *	9.16	60.74	10.24	3.77	9.65	6.41

\*From Torres et al.<sup>(10)</sup>

The comparison of the *Chlorella* products against the *Spirulina* dry powders, showed big differences. First, the protein levels in the *Spirulina* samples showed values up to 60%. Lipid contents were also higher in *Spirulina* than in *Chlorella* samples (8-19%). Crude fiber and carbohydrates values were also quite different. *Spirulina* dry product has been employed as nutraceutical for a long time. *Chlorella* has been commercialized also, in a lower scale. Even mixtures of *Spirulina/Chlorella* products have been offered by some international companies. Zeyfabadi et al (2011) reported that *Chlorella* dry product contained between 33 and 46% of protein, depending on the irradiance (37.5 to 100  $\mu\text{mol photons m}^2/\text{s}$ ), and the employed photoperiod (8:16, 12:12, and 16:8 light/dark hours). Protein content seems to be too high (higher than that reported for *Spirulina* products, about 60%). Bishop and Zubeck (2012) reviewed *Chlorella* dry products characteristics, founding protein, fat, and carbohydrates levels of about 64.5, 10, 15 and 5%. More conservative data reported by Kent et al (2015), establish ash, carbohydrates, lipids and protein contents of 5.7, 24.9, 16.1 and 40%, respectively.

209 The microbiological analysis of the samples was submitted to the analysis proposed by the  
210 Mexican norm and results are showed at Table 3. It is clear that both commercial and home-  
211 made *Chlorella* products are within the levels proposed for a food product. Mesophylic  
212 bacteria were in a concentration  $< 10 \text{ col g}^{-1}$ , as well as yeasts, molds and non pathogenic  
213 coliforms. *Salmonella*, *Shigella* and enteropathogenic *E. coli* were absent from the samples  
214 in both products. That means that both dry products are safe for human consume.  
215 The pigment analysis of the *Chlorella* samples showed the presence of various pigments  
216 and some degradation products (See Fig 1).  
217



218  
219 **Figure 1.** TLC for the commercial (1) and home-made (2) *Chlorella*.

220  
221 **Table 3.** Microbiological analysis for the dry products.  
222



Specification	Maximum for food products	Home made <i>Chlorella</i>	<i>Chlorella</i> vidanat
Mesophylic aerobic bacteria	50,000 col g <sup>-1</sup>	< 10	< 10
Yeasts	10 col g <sup>-1</sup>	< 10	< 10
Molds	10 col g <sup>-1</sup>	< 10	< 10
Non pathogenic coliforms	Negative	< 10	< 10
<i>Salmonella</i>	Negative	Negative	Negative
<i>Shigella</i>	Negative	Negative	Negative
Enteropathogenic <i>E. coli</i>	Negative	Negative	Negative

The analysis of the bands obtained through TLC gave as result, the identification of a group of pigments and their derivatives. Most of the products were common for both the home-made and commercial *Chlorella* samples. As observed in Table 4, both *Chlorella* showed nearly the same pigments and derivatives. Both samples showed the presence of Zeaxanthine (code 22A), a simple carotenol very related with Lutein and  $\beta$ -carotene (code A34). All carotenoids are yellow to red isoprenoid polyene pigments, widely distributed in nature (Liaaen-Jensen and Egeland, 1999).

**Table 4.** Pigment contents in the home-made and commercial samples of *Chlorella*.

Chlorella Sample	Band code	Pigment(s)	Remarks
Home-made	22A	Zeaxanthine	Carotenol
	22B	Pheophytine-like	Chlorophyll degradation product
	22C	Pheophytine a	
	22G	Chlorophyll b	Chlorophyll
	22K	Pheophorbide a	Chlorophyll degradation product
	22L	Pheophytine a	
Commercial Product	34E	$\beta$ -carotene	Carotenol
	22A	Zeaxanthine	
	21B	Pheophytine a	Chlorophyll degradation product
	22B	Pheophytine-like	
	22C	Pheophytine a	
	22G	Chlorophyll b	Chlorophyll
	22K	Pheophorbide a	Chlorophyll degradation product
	22L	Pheophytine a	
	34E	$\beta$ -carotene	Carotenol

Zeaxanthine has been found in Cyanophyta, Prochlorophyta, Rhodophyta and Chlorophyceae division/class as a major (>10%) or minor (1-10%) pigment (Jeffrey et al (1997), and even in Eustigmatophyta as trace pigment (<1%). Also, in both samples was found Chlorophyll a. This is a very common pigment, found in Cyanophyta, Rhodophyta, Cryptophyta, Chlorophyceae, Prasinophyceae, Euglenophyta, Bacillariophyta, Dinophyta, Prasinophyceae and Chrysophyceae division/class. Chlorophylls are green pigments that

form photosynthetic complexes with carotenoids. All chlorophylls are a group of Mg coordination complexes of cyclic tetrapyrroles. Pheophytine a and pheophorbide a were found in both *Chlorella* samples. These molecules are degradation products of Chlorophylls. They have been used as markers for intertidal microbenthos grazing (Cartaxana et al (2003). Kent et al, 2015 reported that *Chlorella* contain about 8.60 mg/g of chlorophyll summation, 1.17 mg/g of carotenoids summation, 0.08 mg/g of astaxantine and 0.19 mg/g of  $\beta$ -carotene. On the other hand, Seyfabadi et al (2011) reported chlorophyll productions for *Chlorella vulgaris* between 7.4 and 13.1 mg/L, depending on the irradiance and photoperiod values employed during the microalgae culture. Regarding  $\beta$ -carotene, these authors mentioned that final biomass contained from 0.02 to 0.07 pg/cell. Finally, Bishop and Zubeck (2102) mentioned that the *Chlorella* product contained 0.086 and 5% of  $\beta$ -carotene and chlorophyll, respectively.

The microalgae pigment contents are of particular interest, since these products show high added values in the market, with prices of 0.1-10 USA\$/kg for chlorophylls (Gonzalez-Delgado et al, 2013) , 882 USA\$/kg for astaxantine (Li et al, 2011), and 300-3,000 USA\$/kg for  $\beta$ -carotene (Hannon et al, 2010). The estimated global markets for pigments are quite huge, i.e., 20, 235 and 275 million USA\$/year for zeaxanthine, astaxanthine and  $\beta$ -carotene, respectively, as examples (Borowitzka, 2013).

Regarding the analysis of lipids, Table 5 show the profile of fatty acids found in both the commercial and home-made samples. As shown, both microalgae samples are rather similar regarding the presence of different lipids, but not regarding the amounts of every fatty acid.

5. Total lipid fatty acid composition of the home-made and commercial *Chlorella* products (% molar).

265

FAME	SAT FAME			MUFA	SAT FAME	PUFA			SAT FAME		
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	Total
Common names	Lauric	Myristic	Palmi-tic	Palmi-toleic	Stea-ric	Oleic	Linole-ic	$\alpha$ -linoleic	Arach-idic	Behe-ntic	-
Home-made <i>Chlorella</i>	NM	0.45	17.49	8.33	12.04	4.67	13.48	23.38	NM	NM	88.41
Commer-cial product <i>Chlorella sorokinia-na*</i>	NM	0.23	18.88	19.15	7.64	13.47	9.11	26.91	NM	NM	88.97
	2.37	0.27	19.72	11.76	1.35	29.85	31.8	NR	0.08	0.03	97.23

\*From Kumar et al.<sup>(3)</sup>. NR- Not reported. NM not measured. SAT FAME-saturated fatty acids, MUFA- monounsaturated fatty acids, PUFA-polyunsaturated fatty acids.

The lipids of the home-made product were composed basically of  $\alpha$ -linoleic acid C18:3 (23.4% in molar basis) and palmitic acid C16:0 (17.5%), followed by linoleic acid C18:2 (13.5%) and stearic acid C18:0 (12%). It can be mentioned that a peak between C16:1 and C18:0 with a retention time of 10.81 was observed. This peak corresponds to an 8.07% and could be presumably the C16:2. Because of the lack of standard, it cannot be confirmed. Total lipids do not sum 100% because of the presence of small peaks with molar percentages less than 1%.

On the other side, the commercial product lipids were composed mainly by  $\alpha$ -linoleic acid C18:3 (26.9%) and palmitoleic acid C16:1 (19.1%), followed by palmitic acid C16:0 (18.9%). Again, a peak between C16:1 and C18:0 with a retention time of 10.83 was observed. This peak corresponds to a 3.31% and could be presumably the C16:2. Because of the lack of



standard, it cannot be confirmed. Total lipids do not sum 100% because of the presence of small peaks with molar percentages less than 1%.

Regarding other works with *Chlorella* strains, Kumar et al (2016) reported the fatty acid composition for *Chlorella sorokiniana* under different modes (fed batch and various dilution rates D values in continuous cultures). Table 5 show the fatty acid content corresponding to *Chlorella* grown at fed batch conditions. Note that the lipids produced were mainly linoleic acid C18:2 (31.8%) and oleic acid C18:1 (29.85), followed by palmitic acid C16:0 (19.7%). Besides, these authors reported the presence of small quantities of lauric acid C12:0 (2.4%), arachidic acid C20:0 (0.08%) and behenic acid C22:0 (0.03%).

From the point of view of microalgae as human food/nutraceutical, it is important to distinguish the amount and type of FAMES present in the *Chlorella* products. The home made product had the highest SAT FAME level, followed by *Chlorella sorokiniana* and the commercial product. The effect of the saturated fatty acids is still controversial. Some authors reported that the ingestion of SAT FAME, except stearic acid is prejudicial for health. Nevertheless, the SAT FAME levels are lower than those reported for other foods such as animal meat. Romero et al (2013) reported SAT FAME percentages of 39, 45, 39 and 43% of the total lipids for *salamin*, *chorizo*, *morcilla* and *chorizo ahumado* (all meat sausages from Argentina).

Regarding the MUFAs, The highest value was for the commercial product, followed by the *Chlorella sorokiniana* sample and our home –made product at the end. It has been reported that diets with healthy amounts of monosaturated fats have health benefits including: 1) decrease risk for breast cancer, 2) reduced cholesterol levels, c) lower risk for heart disease and stroke, d) weight loss, e) less severe pain and stiffness for suffers of rheumatoid arthritis and f) reduced belly fat.

On the other hand, the PUFAs levels for the *Chlorella* samples were higher for the commercial product, followed by the home-made and the *Chlorella sorokiniana* samples, There is substantial evidence that PUFAs induce significant beneficial cardiovascular effects (Ander et al, 2003). Other interesting index is the PUFA/SAT FAME, where the commercial sample had the higher value, followed by the home made and the *Chlorella sorokiniana* products. The higher the index, the better the product. Regarding the PUFA/SAT FAME–stearic acid index, Best value was for the home-made product, followed by the commercial product and *Chlorella sorokiniana* sample, at the end. Finally, the index of MUFA+PUFA/SAT FAME–stearic acid had the best value for the commercial product, followed by the home-made product and the *Chlorella sorokiniana* sample, and the last two were almost identical.

#### 4. CONCLUSION

The production of a home-made product through a strain of *Chlorella* growing in a 21 L agitated tank at outdoor conditions was presented. Some production characteristics such as the irradiance, temperature, pH, conductivities (as a measure of salinity) and other parameters were presented and discussed. The final product was dried and milled, and then, characterized extensively.

The product presented similar characteristics to a commercial *Chlorella* product maybe except in regards to the protein content. The protein content was about 21%, while the commercial *Chlorella* had a protein contain up to 39%. The microbiological characterization indicated that both home-made and commercial products are able to be used as human food or supplement. The analysis of pigments by TLC showed the presence of zeaxhantine,  $\beta$ -

carotene and chlorophyll a, though no quantification was carried out. Other products such as pheophorbide and pheophytin a, were also identified.

Regarding the analysis of lipids, both microalgae samples resulted rather similar regarding the presence of different lipids, but not regarding the amounts of every fatty acid. The lipids of the home-made product were composed basically of  $\alpha$ -linoleic acid C18:3 (23.4% in molar basis) and palmitic acid C16:0 (17.5%), followed by linoleic acid C18:2 (13.5%) and stearic acid C18:0 (12%), while the commercial product lipids were composed mainly by  $\alpha$ -linoleic acid C18:3 (26.9%) and palmitoleic acid C16:1 (19.1%), followed by palmitic acid C16:0 (18.9%).

More work on the optimization of the photobioreactor operation must be carried out, i.e. the effect of the irradiation and temperature over the proteins and pigments concentrations. It can be said that we have produced a dry *Chlorella* product susceptible of being used as nutraceutical or food supplement.

## REFERENCES

1. Ying K, Gilmour DJ, Shi Y, Zimmerman WB (2013) Growth enhancement of *Dunaliella salina* by microbubble induced airlift loop bioreactor (ALB) -The relation between mass transfer and growth rate. *Journal of Biomaterials and Nanobiotechnology* 4:1-9.
2. Loures CCA, Amaral MS, Laite J, Da Ros PCM, De Castro HF, Machado MAG, Silva MB. (2015) Assembling of a bubble-column photobioreactor for the cultivation of a marine microalgae *Chlorella minutissima*. *Proceedings of the IV Congreso Latinoamericano SOLABIA*. Cancun Quintana Roo. Noviembre de 2015.
3. Kumar V, Muthuraj M, Palabhanvi B and Das D (2016) Synchronized growth and neutral lipid accumulation in *Chlorella sorokiniana* FC61ITG under continuous mode of operation. *Bioresource Technology* 200: 770-779.
4. Zhang Q and Hong Y (2014) Comparison in growth, lipid accumulation, and nutrient removal capacities of *Chlorella* sp. in secondary effluents under sterile and non-sterile conditions. *Water Science and Technology* 69(3): 573-578.
5. Mostafa SSM, Shalaby EA, Mahmoud GI (2014) Cultivating microalgae in domestic wastewater for biodiesel production. *Notulae Scientiae Biologicae* 4(1): 56-5.
6. Seyfabadi J, Ramezani Z, Khoeyi ZA (2011) Protein, fatty acid and pigment content of *Chlorella vulgaris* under different light regimes. *Journal of Applied Phycology* 23: 721-726.
7. Bishop WM and Zubeck HM (2012) Evaluation of microalgae for use as nutraceutical and nutritional supplements. *Journal of Nutrition and Food Science* 2(5): 147.
8. Kent M, Wellandsen HM, Mangott A, Li Y (2015) Nutritional evaluation of Australian microalgae as potential human health supplements. *Plos ONE* 10(2): 1-14.
9. Gupta R and Mukerji KJ (2001) *Microbial Technology* Kulbhushan Nangia Ashish Books. Darya Ganj, New Delhi, India.
10. Torres L.G., Gomez y Gomez Y., Bautista E, Corzo L.J. (2017) Production and characterization of a nutraceutical product based on *Spirulina platensis* grown in 200 L bubble columns. *Journal of Microbiology, Biotechnology and Food*. Submitted.
11. AOAC (1997) AOAC. In William Horwitz (Ed.), *Official methods of analysis* (17th ed.). Washington, D.C: Association of Official Analytical Chemists. USA.

382 12. Secretaria de Salud y Asistencia SSA (1994) **Norma Oficial Mexicana NOM-110-SSA1-**  
383 1994, bienes y servicios. Preparación y dilución de muestras de alimentos para su análisis  
384 microbiológico. In spanish only. Mexico.

385 13. Jefferey SW, Montoura RFC, Wright SW. (1997) Phytoplankton pigments in  
386 oceanography. Apendix. Monographs on oceanographic methodology SCOR. UNESCO.

387 14. Bligh EG and Dyer WJ (1959) A rapid method of total lipid extraction and purification.  
388 Canadian Journal of Biochemistry and Physiology 37(8)911-917.

389 15. Slover HT and Lanza E (1979) Quantitative analysis of food fatty acids by capillary gas  
390 chromatography. Journal of the American Oil Chemists Society 56: 933-943.

391 16. Chiu S-Y, Tsai M-T, Kao Ch-Y, Ong S-Ch, Lin Ch-Sh (2009) The airlift photobioreactors  
392 with flow patterning for high-density cultures of microalgae and carbon dioxide removal.  
393 Engineering Life Science 9(3): 254-260.

394 17. Lianen-Jensen S, Egeland ES (1999) Microalgal Carotenoids. In: Chemical from  
395 Microalgae. Cohen Z (Edit). Taylor and Francis. UK.

396 18. Cartaxana P., Jesus B., Brotas V. (2003) Pheophorbide and pheophytin a-like pigments  
397 as useful markers for intertidal microphytobenthos grazing by *Hydrobia ulvae*. Estuarine,  
398 Coastal and Shelf Science 58: 293–297.

399 19. Gonzalez-Delgado A-D, Barajas-Solano A-F, Kafarov V (2013) Obtaining high value  
400 products in a biorefinery topology using microalgae. CT&F. Ciencia, Tecnología y Futuro  
401 5(3):95-106.

402 20. Li J, Zhu D, Niu J, Shen S, Wang G. (2011) An economic assessment of astaxantine  
403 production by large scale cultivation of *Haematococcus pluvialis*. Biotechnology Advances  
404 29: 568-574.

405 21. Hannon M, Gimpel J, Tran M, Rasala B, Mayfield S (2010) Biofuels from algae:  
406 challenges and potential Biofuels 1(5): 763-784.

407 22. Borowitzka M (2013) High-value products from microalgae-their development and  
408 commercialization. Journal of Applied Phycology Doi 10.007/s10811-013-9983-9.

409 23. Romero MC, Romero AM, Doval MM, Judis MA (2013) Nutritional value and fatty acid  
410 composition of some traditional Argentinean meat sausages. Food Science and Technology  
411 33(1): 161-166.

412 24. Ander BP, Dupasquier CMC, Prociuk MA, BSc, and Pierce GN (2003) Polyunsaturated  
413 fatty acids and their effects on cardiovascular disease. Experimental Clinic Cardiology 8(4):  
414 164-172.

415  
416  
417  
418  
419