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Production and Broad Characterization of a *Spirulina platensis* Dry Powder Grown in Bubbled Columns

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Authors' contributions

This work was carried out in collaboration between all authors. Author LT coordinated the research and wrote the first manuscript draft. Authors YL, YGG, EB and LJC participated in the experimental work and helped in the writing/correction of the paper. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this work was to show the feasibility of growing *Spirulina platensis* in bubbled photo-bioreactors in a defined medium and after recovery and drying processes, to obtain a dry powder with food and nutraceutic characteristics such as those obtained by the proximal analysis, lipids and pigments profile, metals content, quelant and radical scavenger, antioxidant and antibiotic properties.
Place and Duration of Study: The work was carried out at Unidad Profesional Interdisciplinaria de Biotecnologia-IPN (Mexico City) facilities during 2017.
Methodology: In this paper, the mass production of microalgae in four 200 L bubbled column addresses growing in alkaline and saline, under ambient conditions and the complete

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characterization of the product in terms of its proximate analysis, metal content and the ability to inhibit growth of some microorganisms. A phytochemicals profile was carried out (qualitative results), the antibiotic activities against Gram negative and positive strains and an analysis of the present lipids and pigments were carried out. Finally, antioxidant and radical scavenging properties of the product were measured.

Results: Photo-bioreactor 1 produced the highest concentration of biomass, although the largest mass of wet weight (757.6 g) and dry (160.8 g), were observed for the photo-photo-bioreactor 4. The photo-bioreactor 4 reached a mass productivity of 2.39 mg $L^{-1}h^{-1}$. On average, 153.35 g dry mass per photo-photo-bioreactor were produced, with a mass productivity of 2.28 mg $L^{-1}h^{-1}$. The obtained product compared well to a commercial one, with similar content of carbohydrates, protein, lipid, fiber, ash and total moisture (%). A phytochemicals profile was carried out (qualitative results). Regarding the Mexican standards, the product is below the recommended values only for ashes and crude fiber. Several alkaline earth and heavy metal values were found in the product. When calculating the intake of an adult weight of 70 kg who ingested 4 g day^{-1} of *Spirulina,* the values are not higher than the recommended intake for an adult by various associations (FAO/WHO, among them). It also found that the product had antibiotic activity against *some Gram-positive and Gram-negative bacteria* strains. It was also identified that the product had antioxidant and chelating activities and an analysis of the present lipids and pigments were carried out.

Conclusion: It can be said that the culture process in the bubbled photo-bioreactors generates a dry product with excellent food and nutraceutic properties which can be employed to solve malnutrition problems that small communities are experiencing in Mexico.

Keywords: Light irradiance; mass production; metals; nutrition supplements; proximate analysis; Spirulina platensis.

1. INTRODUCTION

Spirulina platensis (called from now on only *Spirulina*) cultivation started hundreds of years before in Mexico and China, for the purpose of being used as nutrition supplements [1]. It was reported that this microalga contains significant amounts of carbohydrates, lipids, proteins and vitamins and some minerals of interest in human nutrition. It has also been shown that *Spirulina* contains vitamin A, B_1, B_6, B_{12}, and K, niacin, β-carotene, zeaxanthin, as well as a range of metals in concentrations macro and trace (Ca, P, Fe, I, Mg, Zn, Se, Cu, K, Mn and Na) [2]. This microalgae grows in saline and slightly alkaline environments and has been grown in many different ways for human consumption [3] or to feed fish and crustaceans [4]. The name of nutraceutical food is applied to food that besides providing the necessary elements of the human diet help improve problems such as hyper-tension, blood triglyceride levels, act as antibiotics, etc. *Spirulina* has been regarded as an effective nutraceutical with specific characteristics as hypoglycemic, antihyper-lipidemic, antihypertensive [5,6], antibiotic and even as an agent to prevent the formation of fatty liver [7]. Biomass production or metabolites of interest (i.e., lipids, sugars, proteins, chlorophylls) in *Spirulina*, are determined by several factors, including the medium (nitrogen source), the quantity of light irradiance receiving culture, and ambient temperature [3,8] and the design of the photo-bioreactor.

There are many factors to consider for mass production of *Spirulina* and other microalgae. Among those factors, the selection of the media [9] and the strain, the light irradiation [10] the temperature [11] and the effect of aeration [12]. Parameters such as aeration, mixing and light distribution are much related with the photo-bioreactor selection. Some papers have reported the culture of *Spirulina* in different bioreactors such as raceways (open reactor), tubular photo-bioreactors, bubbled columns and air-lift columns [9,12,13,14]. These photo-bioreactors have different characteristics in terms of the area occupied by the system, the easiness of operation, the mass transfer (mainly the CO_2) inside the reactor, the energy consumption, and the risk of culture contamination. In general terms, open systems such as raceways are very easy to operate and the initial investment is low. Mixing in those reactors is poor and the risk of contamination by other microorganisms is very high. On the other hand, closed bioreactors need smaller areas for construction, they use to be vertical and closed so the risk of contamination is lower [1]. Mixing and mass

transfer issues are more manageable, but initial costs are rather higher than those for raceways.

Ronda et al. [12] reported the use of 20 L bubbled columns for the production of *Spirulina* platensis and specifically with interest in the production of γ-linoleic acid. They reported that the aeration rates in the bioreactor have an important impact on the amount of biomass produced and specifically in the amount of γ-linoleic acid. With a 6-fold increase in the aeration rate, the γ-linoleic acid content of the microalga increased by 69.64% (5.6–9.5 mg· g-1 dry cell weight). In addition, the total fatty acid (TFA) content in dry biomass increased from 2.22% to 4.41%, whereas the algae maintained a constant γ-linoleic acid to TFA ratio within the aeration rate tested. It is difficult to compare the efficiencies of open and closed photo-bioreactors because in the case of the raceways, is very common that productivities are expressed in terms of the reactor open area. As an example, Choong-Jae et al. [14] reported for *Spirulina* cultivation using underground water, mean productivity of 10.5 $gm^{-2}d^{-1}$.

In this paper, the mass production of microalgae in four 200 L bubbled column addresses growing in alkaline and saline, under ambient conditions and the complete characterization of the product in terms of its proximate analysis, metal content and the ability to inhibit growth of some microorganisms. A phytochemicals profile was carried out (qualitative results), the antibiotic activities against Gram negative and positive strains and an analysis of the present lipids and pigments were carried out. Finally, antioxidant and radical scavenging properties of the product were measured.

2. MATERIALS AND METHODS

2.1 Microalgae Cultivation

The cultivation of *Spirulina* was performed in four photo-bioreactors outdoor, under ambient conditions for 14 days in a medium consisting of (in gL^{-1}): NaCl, 5; Na_2HCO_3,10;$CaCO_3$,,0.02; $MgSO_4$, 0.2; KNO_3, 2.0; K_2SO_4, 1.0; $K_2H(PO_4)$, 0.01; $FeSO_4$, 0.005, urea, 0.02. pH was adjusted to 9. Four bubble column photo-bioreactors, manufactured in fiberglass were used. Cultures were aerated 12 hours a day by a perforated ring placed at the base of the photo-bioreactor. The general arrangement of photo-bioreactors and lines are shown in Fig. 1.

Biomass development was followed by reading optical density D.O. Previously a calibration curve DO *vs* dry weight was prepared. The variables were measured throughout the process once a day (about 11 am) was biomass; pH, conductivity, outside temperature, and the photon light irradiance μmol photons $m^{-2}s^{-1}$ were measured. Harvest was carried out by filtering 180 L of algae in a mesh such as those which are typically used for screen printing. Then, a cotton cloth with 180 threads cm^{-2} was used to remove as much water as possible squeezing the sieve. The material thus produced was dried on trays at 60ºC, and it was ground and stored for later proximate analysis. Commercial *Spirulina* Pronat/Ultra (Abastecedora de Productos Naturales SA de CV, Mexico) was characterized with comparison purposes.

2.2 Biochemical Profile

2.5 g of each sample were dry sprayed and 10 mL of distilled water were added and then, samples were sonicated for 10 min. Over time, the samples were filtered and this filtrate was used to perform the phytochemical sieve.

2.2.1 Alkaloids determination

0.5 to 1 mL of the extract was poured in a tube and 5 to 10 mL of hydrochloric acid 10% were added. Tubes were heated to boiling point for 5 minutes; cooled and filtered. This solution was divided into four tubes. a) Tube 1: Dragendorff reaction: a drop of Dragendorff reagent was added, the test was considered positive when an orange precipitate was formed. b) Tube 2: Mayer reaction: a drop of Mayer reagent was added and the test was considered positive when a white precipitate was formed. c) Tube 3: Wagner reaction: a drop of Wagner reagent was added and the test was considered positive when an orange precipitate was formed. d) Tube 4: blank tube.

2.2.2 Determination of flavonoids

0.5 mL of the extract was dissolved in 2 mL of ethanol and was divided into three tubes. Tube 1: blank. Tube 2: Shinoda reaction: 2 drops of concentrated hydrochloric acid were added: a reddish color indicates the presence of chalcones or aurones. If no change occurred, place 10 bits of metallic magnesium. The change of color from orange to red indicates the presence of flavones and if magenta color appears, indicates the presence of flavanones.

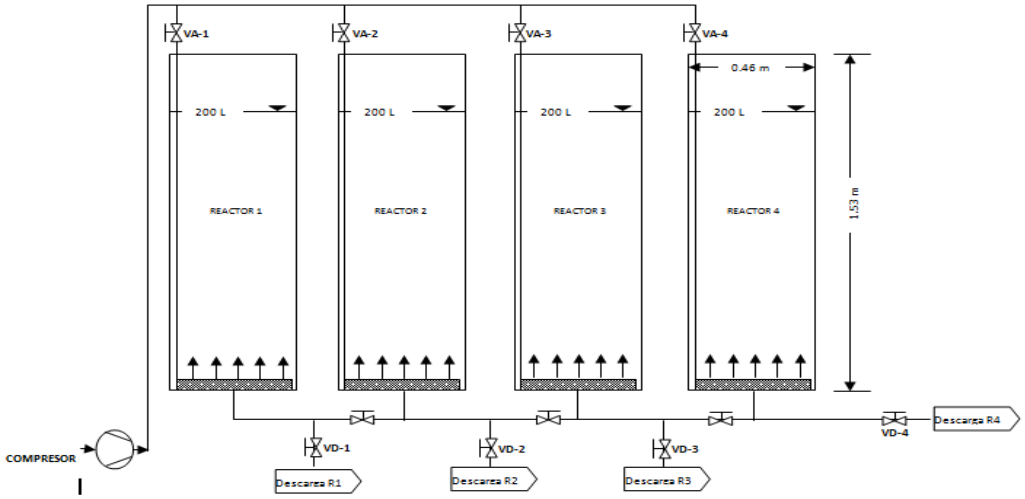

Fig. 1. Photo-bioreactors used in the cultivation of *Spirulina*

Tube 3: reaction of sodium hydroxide 10%. 3 drops of sodium hydroxide were added: yellow to red color indicates the presence of xanthones and flavones, coffee to orange color suggest the presence of flavonoids, reddish purple to blue would suggest the presence of chalcones and anthocyanins.

2.2.3 Determination of cumarines

Reaction with ammonium hydroxide: a portion of the extract was concentrated in a porcelain dish and 0.5 mL of ethanol and 2 drops of concentrated ammonium hydroxide were added. A positive for cumarines is the presence of blue-violet fluorescence.

2.2.4 Tannins determination

2 mL of distilled water and 3 drops of 2% sodium chloride were added to 1.5 mL of the extract. It was heated to boiling for 1 minute. It was cooled and filtered, and the filtrate was divided into four tubes. Tube 1: blank tube. Tube 2: reaction with gelatin, 2 drops of reagent gelatin were added and the presence of a white precipitate indicates the presence of tannins.

2.2.5 Determination of steroids

1 mL of extract was placed in a crucible, it was evaporated to dryness and 3 to 4 drops of chloroform were added. Allowed to dry at room temperature and add 3 to 4 drops of acetic anhydride and 3 to 4 drops of concentrated sulfuric acid. Blue or green color indicates the presence of steroids. Red, pink or violet suggest the presence of triterpenes. A pale yellow is an indication of saturated steroids or triterpenes.

2.3 Proximate Composition

The proximate composition was determined according to the AOAC [15] International methods, namely nitrogen (954.01); fat (920.39); ash (923.03); crude fiber (962.09); humidity (925.09); and total carbohydrates calculated by differences.

2.4 Nutraceutic Properties

2.4.1 Antibiotic properties

In order to evaluate the antimicrobial activity of acetone extract on pathogenic bacteria and yeast, the agar well method was used. Seeding of bacteria and yeast in nutritive broth and Sabouraud broth was performed respectively using the Kirby-Bauer technique in Mueller-Hinton agar [16]. The method consisted of making wells with the aid of a sterile borer of 5 mm diameter on the agar surface, within which is deposited 0.50 mg mL^{-1} and 1 mg mL^{-1} of acetone extract of algae. The bacteria concentration was adjusted to 10^6 CFU mL^{-1}. Plates were incubated for 24 hours at 37ºC [17]. For each microorganism, 100 mL of acetone as a negative control were used. As positive control was used Cephalosporin C (250 mg mL^{-1}), using a strain of *Staphylococcus aureus* as the test organism. Assays were performed in duplicate and the assessment was made by measuring the

diameter of growth inhibition zones around the wells.

2.4.2 Capture of DPPH and ABTS free radicals scavenging activity

A solution of the radical 2,2-diphenyl-1-picrilhidrazile (DPPH) $6x10^{-5}$ M in methanol was prepared. For the assessments, 2ml of DPPH radical and 50 ml of the sample at a concentration of 5 mg mL^{-1} were put together; the mixture was stirred 30 minutes and samples were read at a wavelength 517 nm. [18,19,20]. It was prepared a solution with the reagent 7 mM ABTS and mixed 1:1 with a solution of 2.45 mM potassium persulphate. It was allowed to stand 16 hours in the dark. Then it was diluted with alcohol to obtain an absorbance of 0.72 ± 0.01 at a wavelength of 754 nm. 1,960 µl of the radical ABTS solution were taken and mixed with 40 ml of sample [20,21]. Vitamin C was used as control for both radicals at a concentration of 5 mg mL^{-1}. The uptake for both radicals (%) was calculated as follows:

$$ABTS^{+} \text{ or DPPH scavenging effect} = [(Ac-Am) / Ac] X \quad (1)$$

Where Ac is the absorbance of control and Am is the absorbance of the sample.

2.4.3 Hydrogen peroxide and ferrous ion (Fe^{2+}) chelating activity

One gram per sample was weighed in 50 ml of 70% acetone and sonicated for 30 minutes, and then it was filtered and distilled to remove the acetone [22]. The ability to capture hydrogen peroxide (antioxidant activity) can be measured using the method of Ruch et al. [23]. A solution of hydrogen peroxide (40 mM) in phosphate buffer (50 mM pH 7.4) is prepared. Absorbance at 230 nm is determined. Extracts and control BHT had a concentration of 1.0 mg/ml [23]:

$$H_2O_2 \text{ Uptake (\%)} = [(Ac-Am) / Ac] X100 \quad (2)$$

Ac is the absorbance of control and Am is the absorbance of the sample.

2.4.4 Chelating activity

Ferrozine forms a violet complex red forming chelates with Fe^{2+}. This reaction is inhibited in the presence of other chelating agents, decreasing the absorbance of the sample. The chelating activity is determined using the method of Dinis et al. [24] 0.1 ml of the sample was added at a concentration of 1 mg ml^{-1} to 0.5 ml of a solution of ferric chloride (0.2 mM). The reaction is started by adding ferrozine (5 mM) leaving stand at room temperature for 10 min and absorbance at 562 nm is determined. EDTA was used as control. The percent inhibition of Fe^{2+}-ferrozine complex was calculated as follows [24]:

$$\text{Ion chelating effect } Fe^{+2} (\%) = (1-Am / Ac) X100 \quad (3)$$

Where Am and Ac are the sample absorbance and control absorbance, respectively.

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, only 0.1 g was separated and suspend in 1 mL of acetone in an Eppendorf tube. The tube was closed and maintained from this point on in ice and covered with aluminum foil to prevent light damage. The Eppendorf tube was centrifuged at 16,000 and low temperature (4ºC) during 10 min. The acetone containing the pigments was reserved. About 20 mL of a mixture of petroleum ether (70%) and acetone (30%) was collocated in a large precipitation flask for the thin layer chromatography TLC. See the configuration at Fig. 2. The TLC layers employed were 5x20, 0.2 mm Aluminum oxide N (Macherey-Nagel Co, Germany). Every layer was charged in the bottom side with 10-30 µL of every pigment solution, very slowly and carefully trying to produce a small spot over the layer. The layer was collocated in an inclined way inside the flask and 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 pigment bands start to separate among them. The process was allowed until the solvent was 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 was scratched and suspended in 1 mL of acetone-water solution (80/20%) in an Eppendorf tube, and centrifuged. This process was repeated if still some Aluminum oxide particles

were evidently floating on the solvent. Every pigment solution was read in a UV/Vis spectrophotometer (Perkin-Elmer Lamda 25). The profiles of abundance vs. wave length are stored and compared against those profiles previously reported in Jeffrey et al. [25].

2.6 Lipids Profile

Lipids were extracted following the method of Bligh and Dyer [26] and then, were saponified and methylated by the method of Slover and Lanza [27] 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 Biomass Growth

The development of the four cultures is shown in Fig. 2 as the D.O. of the culture over 14 days. Although the curves for the four cultures were similar, it was observed that at day12 the amount of biomass started to decrease. This situation could be due to cold days for those days, cloudy days and rainy daily presence was observed.

Table 1 shows the summary of the characteristics of each culture. As shown, photo-bioreactor 1 produced the highest concentration of biomass, although the largest mass of wet weight (757.6 g) and dry (160.8 g), were observed for the photo-bioreactor 4 at 14 days of process. Thus, the photo-bioreactor 4 showed the higher mass productivity reached 2.39 mg $L^{-1}h^{-1}$.

On average, 153.3 g dry mass occurred, with a mass productivity of 2.3 mg $L^{-1}h^{-1}$ and light irradiance values and room temperature for a day (light phase) are shown in Table 2. As shown, in one day, they may be light irradiances on the order of up to 2,500 μmol of photons $m^{-2}s^{-1}$ to 15 hrs. In that specific day the sun rose at 7 am and set at 7 pm (summer horary, Mexico City).

As shown in the Table 2, the optical density of the culture increases from day 1 to day 11, when reached the higher value (0.904). Also, daily light irradiance values are high (i.e., greater than 1,100 μmol photons $m^{-2}s^{-1}$) during the first 8 days of culture. From the 10th day, low light irradiance values were observed (185-700 μmol of photons $m^{-2}s^{-1}$). Weather conditions. Clear skies were observed from day 1 to day 11. The following days, a cloudy sky and even showers were observed. Operating temperatures were on average 24ºC, while the maximum was observed on day 8 (32ºC). The conductivity of the medium did not show significant trend, increasing and decreasing slightly, averaging 22.6 mS. Medium pH was saline and alkaline, with an initial pH value of 9 units. By the 12th the value found was 10.3 and the trend was always to increase along the process.

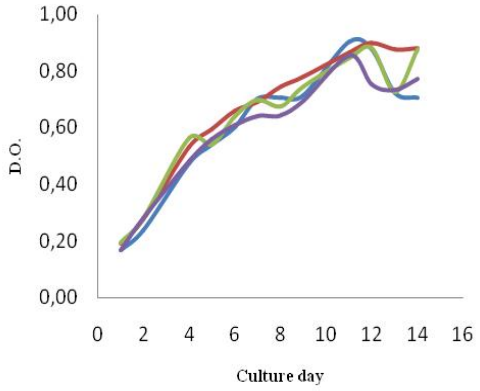

Fig. 2. Growth of *Spirulina* in the photo-bioreactors 1 (blue), 2 (red), 3 (green), 4 (purple)

3.2 Biochemical Profile

The first *Spirulina* powder characterization consisted in searching the presence of compounds that belong to the following groups: alkaloids, flavonoids, cumarines, tannins and steroids. Assessments were carried out by triplicate using the home-made product and the commercial *Spirulina* samples.

Table 3 shows the results of biochemical assessments for both home-made and commercial *Spirulina* aqueous extracts. It is clear that the biochemical profiles for both samples were identical. Regarding the identification of alkaloids, the Mayer reaction did not showed presence of activity, but the Wagner and Dragendorff reactions did it. The Dragendorff reaction accused a high presence of alkaloids in the samples. Antonisiamy and Eahamban [28] found alkaloids in the macroalgae *Dictyotabart ayresiana* Lamour, when using chloroform, benzene, and petroleum ether (cold and soxhlet methods) for extraction. On the other hand, Sudha et al. [29] reported the presence of

Table 1. *S. platensis* culture parameters obtained in the four 200 L photo-bioreactors 1-4

Photo-bioreactor	X_{max}g L^{-1}	W_{wet}g	W_{dry}g	Masic production mg $L^{-1}day^{-1}$
1	0.904	674.6	137.5	48.9
2	0.898	734.8	156.0	55.7
3	0.88	749.7	159.1	52.3
4	0.854	757.6	160.8	57.4
Average	0.884	729.175	153.35	53.6

Note: X_{max}g L^{-1}, mass productivity; W_{wet}, biomass wet weight; W_{dry}, biomass dry weight.

Table 2. Temperatures, light irradiance, and absorbance characteristics of the days of culture (at 11 am). Specific data for the photo-bioreactor 1

Day	D.O.	pH	Conductivity mS	T°C	Light irradiance μmol photons $m^{-2}s^{-1}$	Atmospheric conditions
1	0.169	9.03	22.6	23	1296	Clear
2	0.239	9.17	23.1	29	1335	Clear
4	0.475	9.54	22.3	25	1302	Clear
5	0.538	9.71	21.5	24	1271	Clear
6	0.603	9.78	21.2	24	1250	Clear
7	0.702	9.92	21.8	22	1310	Clear
8	0.706	9.94	21.8	32	1104	Clear
9	0.714	10.03	23.0	20	NM	Clear
11	0.904	10.13	21.3	20	380	Clear
12	0.878	10.32	21.9	23	700	cloudy
13	0.722	NM	NM	17	185	cloudy
14	0.715	NM	NM	NM	NM	Cloudy and rainy
Aver	-	-	22.6	24.2	1,013	-

NM-not measured

alkaloids in *Spirulina platensis* and related it with the antimicrobial activity of *Spirulina* against some microorganisms, including *Echerichia coli, Klebsiella pneumonia,* and *aeruginosa, Proteus sp, Empedobacter sp* and *Staphylococcus aureus*.

On the other hand, flavonoids were not identificated neither by the Shinoda reaction, neither by the NaOH 10% assessment. Definitively this family of compounds is not present in the *Spirulina* samples. Flavonoids are an interesting group of compounds with biological activity. Recently, Goiris et al. [29] reported the presence of flavonoids in different microalgae from evolutionary lineages. They showed the presence of flavonoids using UHPLC-MS/Ms equipment in *Phaeodactylum tricornutum, Diacronema lutheri, Porfidium purpureum, Haematococcus pluviales, Chlorella vulgaris, Tetraselmis suecica* and *Arthospira platensis.*

In the same Table 3, it is shown the presence of cumarines in the sample, by the $NH_4(OH)$ reaction. As far as we know, there are no reports of cumarines presence neither in macroalgae [28] or microalgae [30]. This last work analyzed the presence of coumarin in *Phormidium fragile, Lyngbya limnetica, Scytonema bohnerii and Calothrix fusca*, but coumarine was always absent. Coumarines can be employed as anticoagulants. Godovindappa et al. [31] have related the presence of coumarin in the plant *Sonchus oleraceus* with blood anticoagulant activity, and they suggest the use of this plant compound to substitute synthetic products such as warfarin.

Regarding the tannins in the *Spirulina* samples, it can be said that they are present due to the positive results of the gelatin test. Kavitha and Palani [32] reported absence of tannin in *Chlorococcum humicola* microalgae, while Sharatchandra and Rajashekhar [30] reported the presence of tannins in *Phormidium fragile, Lyngbya limnetica, Scytonema bohnerii and Calothrix fusca.*

Finally, the presence of sterols in both samples is evident thanks to the Lieberman-Bouchard positive reaction. Sharatchandra and Rajashekhar [30] reported the presence of steroids in samples of *Phormidium fragile, Lyngbya limnetica* and *Scytonema bohnerii.*

Table 3. Biochemical profile for home-made and commercial *Spirulina* samples. Aqueous extracts

Metabolite	Reaction	Photo-bioreactor 1 *Spirulina*	Commercial *Spirulina*
Alkaloids	Dragendorff	++	++
	Wagner	+	+
	Mayer	-	-
Flavonoids	Shinoda	-	-
	NaOH 10%	-	-
Cumarines	$NH_4(OH)$	+-	+-
Tannins	Gelatin	+-	+-
Steroids	Lieberman-Bouchard	+-	+-

Symbols: – represents absence of activity, +- represents a moderate presence, + represents a definitive presence of activity and ++ means a strong presence of such activity.

Nevertheless, Khavita and Palani [32] reported that *Chlorococcum humicola* did not contain tannins. Finally, Luo et al. [33] summarized the presence of different phytoesterols in *Isochrysis galbana, Nannocloropsis gaditana, Nannochloropsis sp., Phaeodactylum tricornutum, Pavlova lutheri,* and *Tetrasellmis sp.* Phytoesterols are very important secondary metabolites present in animal and vegetal cellular membranes as they are related with regulation of membrane fluidity and permeability. Humans cannot endogenously synthesize phytoesterols, and have to gain them from diet. Since the mid-90`s phytoesterol products have been commercialized as nutraceuticals or pharmaceuticals with the ability of lowering the blood cholesterol level (such as cytellin).

3.3 Proximate Analysis

Regarding the proximate analysis of the microalga using the batch from the photo-bioreactor 1, the measured composition is listed in Table 4, in comparison with a commercial product. Spirulina for human consumption is regulated by the NMX-F-508-1988 [34]. The commercial product and the product of photo-bioreactor 1, had a protein content over 60%. As for carbohydrates, home products presented a 9.16% compared to the demands of the standard is still low (Min 13%) and the commercial product exceeded that value (16.6%). Regarding lipids, *Spirulina* from photo-bioreactor 1 presented a percentage of 10.2% compared to the Mexican standard (minimum 6%) and the commercial product (8.3%), which is good for the product. The total organic nitrogen had a value of 7.56%, compared to 9.6% of the commercial product (16.4%). As for the crude fiber, none of the products was below the maximum value of 0.9% of the Standard. The value of ashes was also a little high for our product (9.65%) compared to the standard (up 9%). Finally, the moisture of the product from the photo-photo-bioreactor and the trade, passed the standard moisture (6.4 and 7.4%, respectively), which provides up to 10%.

Colla et al. [11] reported the production of *Spirulina* biomass with different protein, lipid and phenolics content regarding the culture temperature (30 or 35ºC), and the level on $NaNO_3$used in the culture. For example, *Spirulina* biomass cultured at 30ºC had less protein contents than those obtained at 35ºC (57.6% or a culture grown at 30ºC in respect to 65.4% for a culture grown at 35ºC, at the same $NaNO_3$ concentration, i.e. 2.5 g L^{-1}).

Table 4. Proximal analysis for *Spirulina* produced in the photo-bioreactor 1 compared with a commercial product

Sample	Carbohydrates (%)	Protein (%)	Lipids (%)	Raw fiber (%)	Ashes (%)	Humidity (%)
Spirulina Photo-bioreactor 1	9.16	60.74	10.24	3.77	9.65	6.41
Spirulina Pronat/Ultra	16.63	60.75	8.30	4.41	2.45	7.44
Mexican Norm	Min 13	Min 60	Min 6	Max 0.9	Max 9	Max 10

Regarding the lipid contents, a higher temperature promoted in general higher lipid content also. As an example for the two mentioned temperatures (30 and 35ºC) and the same $NaNO_3$ concentration, the biomass lipids contents were of 8.1 and 0.9 %, respectively. Interestingly, the μ_{max} value obtained for the two temperatures was higher for the lower temperature level (μ = 0.074 and 0.054 day^{-1}, respectively).

3.4 Metals Content

The two samples of dried spirulina were analyzed for metal content. Appropriate digestions were performed and the content of metals in mg/kg of dry biomass was determined. As can be seen in Table 5, commercial Spirulina and the home-made product have many similarities. None of the products contains sodium, but in terms of K, our product has 14,053 mg kg^{-1}. In the case of Ca, our product has a concentration of 2,435 mg kg^{-1} while the commercial product did not show presence of that metal. Comparing the values of Mg in the commercial product (1,905) and home product (5,683) both in mg/kg, it can be seen that there is a large amount of salts in the product generated in the photo-bioreactor. Perhaps it is because many of the medium salts were left in the product after filtration, so many metals occur in high amounts. One element to be analyzed is the Al, which did appear in the commercial product, since in many cases Al salts are used for coagulation-flocculation of microalgae.

Al concentration in the home-made product is low (108 mg kg^{-1}) compared with the commercial product (477 mg kg^{-1}). In the case of home product, no coagulant-floculant was added. It should be noted that nowadays the consumption of Al, is related to Alzheimer's disease, so it is necessary to avoid intake of this important metal. Mn stimulates the adsorption of Ca in the bones and some in the form of salts (carbonate, sulfate, citrate, etc.), so is included in some vitamin supplements. The home-made product and the commercial one, showed Mn concentrations of 23 and 8 mg kg^{-1}, respectively. Our product had a content of 242 mg kg^{-1} of Sr compared the commercial product presented only 4 mg kg^{-1}. There are no explanations for that fact.

It is noteworthy that the alkaline earth metals (Na, K, Ca, Mg, Sr and Rb) are abundant in the earth's crust and are not hazardous to health. Regarding Fe, our product and the commercial one presented high amounts of it (1,109 and 946 mg kg^{-1}, respectively). It can say that Fe is a very common element, but in high concentrations can cause damage. Doses of 20 to 60 mg kg^{-1} body weight can cause acute poisoning. Fe is recommended for anemia problems and consumed as an organic derivative, preferably.

The last evaluated metals (Co, Ni, Zn and Cd), are known as heavy metals and may have trouble when ingesting in high doses. For example zinc has many functions and is involved in the transaction in about 300 enzymatic reactions in the body. Like all other nutrients, they may have very low levels (nutritional deficiencies), relatively low levels (sub-optimal levels), appropriate levels and toxic levels. The recommended daily dose of zinc ranging up to 10 to 12 mg day^{-1} and the maximum dose for the general population is around 40 mg day^{-1}. Our product presented a Zn concentration of 5 mg kg^{-1}, compared with the commercial product, with 22 mg kg^{-1}. Cobalt is a necessary complement to healthy development. Cobalt is an element that is classified as a heavy metal, need vitamin B_{12}. It has also recently been identified as an integral part of the production of blood cells. Our product introduced 2 mg/kg dry weight, while the commercial product was not found. Although the rule does not even considered to heavy metals, the Association of Official Analytical Chemists (AOAC) suggests the following maximum levels (mg kg^{-1}): Lead, 4; Cd, As 0.5, 2.5; 0.05 Hg [34]. Muniz-Moreira et al. [35] cultivated Spirulina as a source of essential minerals and other nutrients for Brazilian necessities. They found macro-minerals (in mg kg^{-1}) such as Ca (15.10), Na (14.65), and K (14.03). On the other hand, they detected Fe (965), P (8.7) Mg (4.7), S (6.7), Mn (106), Zn (35.4), Cu (11.9), and Cr (4.0). Regarding the proximal analyses the measured a content of protein (65%), ash (12.0%), carbohydrate (7.5%), lipid (7.0%) and crude fiber (0.95%).

3.5 Antibiotic Properties

As for the activity of dry *Spirulina* as an antibiotic, it is important to remember that an antibiotic is best if the inhibition halo produced is wide, corresponding to the lowest concentration. For home product (Table 6), it is noted that inhibits the growth of all microorganisms assessed except *Yardia enterolitica* and *Bacillus subtilis* (for the methanol extract and 10 mg strength), compared to the commercial product which was not inhibitory for *Klebsiela pneumoniae*, Yardia

Table 5. Contents of metals in products, mg kg^{-1}. Column 3 shows the intake of an adult of 70 kg (supposing a 4 g daily intake) and the recommended dose

Metal	Spirulina Pronat/Ultra	*Spirulina* Photo-bioreactor 1	Adult ingest (mg day^{-1})	Recommended dosis (mg day^{-1})
Be	ND	ND	0	-
Li	4	6	0.024	-
Na	ND	ND	0	-
K	ND	14,053	56.21	4,700[e]
Ca	ND	2,435	9.74	2,500[e]
Mg	1,905	5,683	22.73	350[e]
Sr	4	242	0.968	-
Rb	354	ND	0	-
Ba	1	22	0.088	-
Cs	ND	ND	0	-
Al	477	108	0.432	70.0[a]
Pb	ND	ND	0	0.25[b]
As	ND	ND	0	-
Bi	ND	ND	0	-
Se	ND	ND	0	0.400
Cr	40	17	0.068	0.05-0.2[c]
Mn	23	8	0.032	9.8[d]
Fe	1,109	946	3.78	45
Co	ND	2	0.008	-
Ni	6	ND	0	1.4[d]
Zn	22	5	0.02	15[e]
Cd	ND	ND	0	0.07[a]

FAO/WHO (1989[a], WHO (1993)[b], NRC (1989)[c], EPA (2001)[d], RDA[e].Data from Santos [36]

Table 6. Inhibition hales (mm) for *Spirulina* (commercial and photo-bioreactor 1 products)

Activity against microorganism	*Spirulina* Pronat/Ultra				*Spirulina* Photo-bioreactor 1			
	acetone		methanol		acetone		methanol	
Concentration, mg	10	5	10	5	10	5	10	5
Klebsiela pneumoniae	-	-	-	-	0.7	-	-	-
Yardia enterolitica	-	-	-	-	-	-	-	-
Bacillus cereus	1.4	0.9	-	-	2	1.2	1	0.8
Enterobacter agglomerans	1.2	0.9	-	-	1	1	-	-
Escherichia Coli	1.2	-	-	-	0.7	-	-	-
Salmonela thypi	1.7	1	-	-	1.1	0.9	-	-
Klebsiela rhinoscleromatis	1.7	1.1	0.7	0.7	1.1	0.9	0.8	-
Staphylococcus aureus	1.1	0.75	-	-	0.8	-	-	-
Listeria monocitogenes	0.7	-	-	-	0.7	-	-	-
Shiguella disenteriae	1.4	1.1	0.8	-	1.5	1.2	0.8	0.8
Candida albicans	1.4	0.9	-	-	1	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-	-

enterolitica and *Bacillus subtilis*, under the same conditions (see Table 6). In general, the metabolites dissolved in acetone showed always higher antibiotic activity than those found in the methanolic extracts. The higher the extract concentration (5 o10 mg), the higher the antibiotic activity.

3.6 Antioxidant and Chelating Activities

Table 7 shows the antioxidant and chelating activities of the commercial algae and home products. It can be seen that the commercial and home Spirulina had antioxidant activity (expressed as H_2O_2 scavenging activity) of a

49.9 and13.1% respectively compared to BHT (31.3%). In addition, ferrous ion chelating activity of the same products was 30.7 and 27.5% respectively, compared to EDTA (93.6%). These activities are greatly appreciated for a nutraceutical product. The scavenging activity of DPPH was 6.2 and 10.9% and $ABTS^{+}$14.3 and 28.6% with respect to vitamin C (100%) for both radicals.

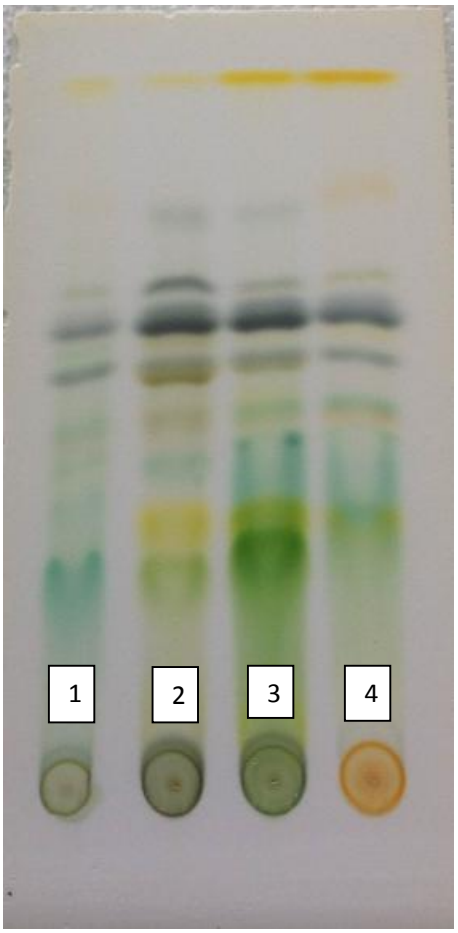

Fig. 3. TLC layer for the commercial *Spirulina* product (1) and three home-made products (2,3,4)

Martinez Palma et al. [37] studied the antioxidant and chelating activity of protein hydrolysates from *Spirulina* obtained by simulated gastrointestinal digestion (enzymatic hydrolysis). They found that the *Spirulina* hydrolysates showed antioxidant and chelating activities, even after hydrolysis degrees equivalent to 31.4 and 36.7%.

DPPH radical scavenging activity of hydrolysates had values up to 60%, while ABTS-scavenging capacity reached values as high as 2.5mM Trolox. On the other hand, the antioxidant activity had a maximum value of 70 (%) measured as the inhibition of b-carotene and chelating activities for Fe^{+2} and Cu^{+2} were as high as 55 and 65%, respectively [37].

3.7 Pigments Profile

The extraction of pigments from the home-made and the commercial *Spirulina* products was carried out. Fig. 2 show the TLC for the mentioned assessment. The results for the pigment identification in the dry *Spirulina samples* were as follows (See Table 8). The commercial and the home made products were rather similar in pigments. The commercial product contained a band corresponding to zeaxanthin mixed with chlorophyll a (they were not properly separated in the TLC). Besides, pheophitine, zeaxanthin and b-carotene were identified. Differences between band colors among samples (commercial/home-made) mean different pigment types. Pheophithine is a degradation product of chlorophylls (gray color). These antioxidant activities are related with the *Spirulina* content regarding non-enzymatic antioxidants such as phenols, pigments i.e., chlorophyll, pheophitine, carotene, phycocyanine, zeaxanthine, etc and antioxidant enzymes (superoxide dismutase, catalase and peroxidase) as studied by Ismaiel et al. [38]. The DPPH, ABTS and H_2O_2 scavenger assessments are indirect probe of *Spirulina* powder antioxidant activities.

Table 7. Antioxidant and quelant activities of *Spirulina* samples

Sample	Peroxide scavenger activity (%)	Quelant activity (%)
Spirulina Pronat/Ultra	49.9+/-6.4	30.75+/-4.3
Spirulina Photo-bioreactor 1	13.1+/-3.2	25.75+/- 5.5
BHT (butylhidroxitoluene)	31.3 +/-4.1	-
EDTA	-	93.6+/-1.9

Table 8. Pigment contents in the commercial and home-made samples of *Spirulina*

Pigment	code	*Spirulina* photo-bioreactor			
		Commercial product	1	2	3
Zeaxanthine + Chlorophyll a	34A		x	x	x
Pheophitine	31G	x	x	x	X
Zeaxanthine	32H	x	x	x	x
β-Carotene	34E	x	x	x	x

Table 9. Total lipid fatty acid composition of the home-made and commercial *Spirulina* products (% molar)

FAME	SAT FAME		MUFA	SAT FAME	SAT FAME	MUFA	PUFA			Total
	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C20:2	
Common names	myristic	palmi-tic	palmi-toleic	hepta-decanoic	stea-ric	oleic	linoleic	α-linoleic	eicosadi-enoic	-
Home-made *Spirulina* 1	<1.0%	43.17	10.16	NM	1.69	1.71	17.88	22.21	NM	96.82
Home-made *Spirulina* 2	<1.0%	45.14	67.06	NM	1.46	1.24	18.88	21.91	NM	94.69
Home-made *Spirulina* 3	<1.0%	43.23	7.58	NM	2.42	1.03	16.13	20.29	NM	90.68
Commercial product	<1.0%	41.66	11.25	NM	1.53	2.95	16.27	17.17	NM	90.83
*Arthrospira platensis**	NR	54.78	2.47	0.23	1.24	3.47	24.94	12.63	0.23	99.99

3.6 Lipids Profile

Regarding the lipids profile, Table 9 shows the lipid compositions of the 3 home-made *Spirulina* products, the commercial product and one *Spirulina* product reported by Almahrouqi et al. [39]. The three home made products resulted very similar among them. The most common lipid present was the palmitic acid C16:0 was also abundant in percentages between 43.17 and 45.14%. After that, a-linoleic acid C18:3, in molar percentages between 20.29 and 22.12%. Then, the linoleic acid C18:2 was the following in abundance, in percentages between 16.13 and 18.18%. There was also palmitoleic acid C16:1, oleic acid C181, and finally, the presence of stearic acid C18:0.

The commercial product was slightly different with a major percentage of palmitic acid C16:0 (41.6%), followed by a-linoleic acid C18:3 (17.17%) and then linoleic acid C18:2 (16.27%). Small percentages of palmitoleic C16:1, oleic C18:1 and stearic C18:0 acids were also present.

The *Spirulina* reported by Almahrouqi et al. [39] was more different to the previous products, since the major percentage was also palmitic acid C16:0 (54.78%), followed by linoleic acid C18:2 (24.94%), and then a-linoleic acid C18:3 (12.63%). Small percentages of palmitoleic C16:1, oleic C18:1 and stearic C18:0 acid were also present, but also heptadecanoic acid C17:0 in a very low percentage (0.23%).

In all cases the total percentages of fatty acids was not 100% (from 96.8 to 99.9%, due to the presence of small amounts of other fatty acids not identified. There are some indexes that indicate the quality of the microalgae samples from the point of view of their use as food/nutraceutics. The total SAT FAME index means the amount of saturated FAMEs present, since this group has been very controversial. Some authors reported that the ingestion of SAT FAME, except stearic acid is prejudicial for health. The products with the higher level of SAT FAMEs were the product reported by Almahrouqi et al. [39] followed by the home-made products and the commercial product at the end.

The monounsaturated FAMES, called MUFAS, have been reported as beneficial to human health. In particular, 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. The products with higher MUFAs concentrations were the commercial product, followed by the home-made products and the *Spirulina* reported by Almahrouqi et al. [39] at the end.

The polyunsaturated FAMEs called PUFAs were also present in the Spirulina samples, in higher percentages in the home-made products followed by the commercial one and the product reported by Almahrouqi et al. [39] at the end. There is substantial evidence that PUFAs induce significant beneficial cardiovascular effects (Ander et al. 2003).

The ratio PUFA/ SAT FAME is an interesting index, and this value was higher for the home made products. Becker (1993) summarized from different papers that commercially important fatty acids are linoleic acid (C18:2), γ-linoleic acid (C18:3), dihomo-linoleic acid (C20:3), arachidonic acid (C20:4) and eicosapentanoic acid (C20:5). Among them, the *Spirulina* strains contained at least a good amount of linoleic acid.

4. CONCLUSION

As for the production of *Spirulina* platensis outdoors, good production levels were achieved in 14 days, with maximum biomass of 160 dry weights and maximum productivity of 57.3 mg $L^{-1}day^{-1}$. The obtained product compared to a commercial one, with content of carbohydrates, protein, lipid, fiber, ash and total moisture of 9.1, 60.7, 10.2, 3.7, 9.6, and 6.4%, respectively. Regarding the International Standard, is below the recommended crude fiber and ashes content. Several alkaline earth and heavy metal values were found in dry products. When calculating the intake of an adult weight of 70kg ingested 4 g/day of Spirulina, the values are not nearly higher than the recommended intake for an 70 kg adult by various associations (WHO, EPA, NRC, FAO, etc.). It also found that the product had homemade antibiotic activity against *K. pneumoniae, B. cereus, E. agglomerans, E. coli, S. thypi, K. rhinoscleromatis, S. aureus, L. monocytogenes, S. dysenteriae and C. albicans*. It can be seen that the commercial and home–made *Spirulina* had antioxidant activity (expressed as H_2O_2 scavenging effect) of 49.9 and 13.1%, respectively, compared to BHT (31.3%). In addition, ferrous ion (Fe^{2+}) chelating

activity of the same products were 30.7 and 27.5% respectively, with respect to EDTA (93.6%). $ABTS^+$ radicals are more reactive than DPPH. As a resume, it can be said that the culture process in the bubbled photo-bioreactors generates a dry product with excellent food and nutraceutic properties which can be employed to solve malnutrition problems that small communities are experiencing in Mexico.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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