Journal of Agricultural Science and Technology B 10 (2020) 298-307
doi: 10.17265/2161-6264/2020.05.004

Physicochemical and Thermal Characterization of the *Spirulina platensis*

Anny Manrich[1], Juliano Elvis de Oliveira[2], Maria Alice Martins[1] and Luiz Henrique Capparelli Mattoso[1]

1. Nanotechnology National Laboratory for Agriculture (LNNA), Embrapa Instrumentação, Rua XV de Novembro 1452, São Carlos 13561-206, SP, Brazil

2. Engenharia de Materiais, Departamento de Engenharia, Universidade Federal de Lavras, Av. Doutor Sylvio Menicucci, 1001, Lavras 37200-900, MG, Brazil

Abstract: Cyanobacterium *Spirulina platensis* is commercially produced as a nutrient source for food, animal feed and pharmaceutical industries, and is also explored in other applications in areas such as material sciences, materials engineering and for the production of biofuels and biochemicals. Due to the increasing interest in the use of this microalga, a complete characterization was intended, as to provide data to the insufficient literature. In this work, various techniques were used for thermal (thermogravimetric analysis (TGA)/derivative thermogravimetry (DTG), differential scanning calorimetry (DSC)), structural (scanning electron microscopy (SEM) and wide-angle X-ray diffraction (XRD)) and chemical (atomic absorption spectroscopy (AAS), attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), energy dispersive X-ray spectroscopy (EDX)) characterization of *Spirulina* cultivated in Brazil. Results have shown that in addition to the high quantity of protein (over 50%), Brazilian *Spirulina* is a source for carbohydrates (33%) and also has good thermal stability up to 200 °C. The pigment protein Phycocyanin could be identified by FTIR and ultraviolet-visible (UV-vis) spectroscopy. Results show favorable properties of *Spirulina* as a source for new materials and biomass.

Key words: *Spirulina platensis*, characterization, blue-green algae, chemical composition, amino acids.

1. Introduction

The development of strategic materials that meets society expectations, made of ecologically and economically suitable renewable resources has been for many researchers a challenging task. Because of the distinct natures and different origins of the matter used, the decision prior which applications are more suitable for each feedstock is critical where not only its general characteristics are relevant, but also its individual features, determined by its cultivar, geographical precedence and manage. From this point of view, complete characterization studies of different resources and biomass, based on reliable techniques are crucial. The characterization will direct one to decide over the appropriate applications for a specific resource and in many times outlines, if it can be safely used as food source. *Spirulina* is a blue-green algae belonging to cyanobacter, a group of photosynthetic microorganisms, which, although unicellular, grow in grouped filamentous forms. Increasing interest in the study of this microalga is due to its high application potential [1-3]. The two most important species of *Spirulina*, *Spirulina maxima* and *S. platensis*, can be harvested in water, dried and used as food. *Spirulina* is one of the most important industrially cultivated microalgae [2]. Studies indicate that in addition to its 50% to 70% protein content in dry matter, it has essential amino acids, essential lipids, unsaturated fatty acids, important vitamins and minerals of high nutritional value [1-4]. *Spirulina* has also been used to treat many diseases due to therapeutic characteristics, such as strong anti-inflammatory and antioxidant properties [3, 5-7] and has been recommended as a

Corresponding author: Anny Manrich, Ph.D., post-Doc, research field: food science and technology.

nutritional supplement to improve the performance of athletes [4]. Besides being used for supplementing the diet of humans and animals, an important application area of *Spirulina* is waste water treatments, as *Spirulina* shows a good binding capacity to ions and oils which may be used to purify water contaminated with metals, oil and lubricants [8].

Research areas for *Spirulina* include energy and obtaining bioactive substances to use in drugs and cosmetics [3, 9] whereas it is an important source of C-Phycocyanin, which has been widely used in commercial applications in the food and cosmetic industry as a natural blue dye [10]. Another possible application is in the area of materials, the production of edible films and biodegradable packaging, replacing the use of materials derived from petroleum. This is because protein-based films tend to have higher barrier properties than lipid and carbohydrate-based biodegradable films [11, 12]. Both the physical and chemical characterization of commercial and non-commercial *Spirulina* has been carried out by some authors [6, 12, 13].

Due to the growing on consumption and on interest in commercial exploitation of *Spirulina*, the objective of this work was to assemble, in a single work, a wide characterization of Brazilian S*pirulina*, which could provide information on the physical and chemical properties of this microalga targeting new applications [1, 3, 4].

In this paper, *S. platensis* cultivated in Brazil was characterized in terms of the chemical composition, including amino acids, ultraviolet-visible (UV-vis) and Fourier transform infrared (FTIR) spectroscopy, X-ray diffractometry, elemental analysis by energy dispersive X-ray spectroscopy (EDX) and atomic absorption spectroscopy (AAS), thermal analysis and scanning electron microscopy (SEM), to impart important information aiming at new applications. Results of this complete characterization show that the *S. platensis* cultivated in Brazil is a strategic biomaterial, source of proteins, lipids and various carbohydrates; in addition it has a good thermal stability, therefore fitting the development of food and feed supplementation innovations, biodegradable composite films, pharmaceutical developments and nutritious packaging material.

2. Materials and Methods

2.1 Materials

Commercial *S. platensis* (Fazenda Tamanduá) in dried powder form was purchased from a local market in João Pessoa, PB, Brazil (Fig. 1). Carbohydrate standards of high performance liquid chromatography (HPLC) grade were purchased from Sigma (USA). Other reagents were of analytical degree.

Fig. 1 *Spirulina platensis* in form of dried powder commercialized by Fazenda Tamanduá.

2.2 Methods

Extractives were analyzed in two steps aimed at the separate characterization of lipids and chlorophylls (or non-lipid extractives). As it is a low polarity solvent, petroleum ether extracts lipids and certain lipophilic vitamins from *Spirulina*. The most used method for extracting total lipids is the Folch [14] extraction procedure, using 2:1 chloroform:methanol. Pohndorf *et al.* [15] compared the yield and the composition of *Spirulina* extracted lipids by cold (with chloroform/methanol) and hot (using hexane) extraction methods. The maximum yield was achieved by the cold procedure (5.8 ± 0.6 g/100 g), approximately three times more than when performing hot extraction. However, due to the toxicity of these solvents and the higher energy costs for the purification of the extracted lipids due to also higher polarity of the solvents mentioned [15], a polar solvent was used in the present work. For a routine total lipid determination, also known as ethereal extraction analysis, petroleum ether or diethyl ether is mostly used [16]. Consequently, chlorophyll can be selectively extracted by acetone [17]. Sequential solvent extractions using petroleum ether and acetone were used to determine the total lipids and total chlorophyll of *Spirulina*. Extractions were made using solid-liquid extraction with a Soxhlet extractor. For the assay, 2 g of the sample was wrapped in paper filters and positioned on the extractor. Afterwards, 250 mL of the solvent was placed in previously weighed flasks for a continuous extraction routine of 8 h with 10 cycles per hour. After complete removal of the solvent in an oven at 50 °C, the flask was weighed and the extractives were calculated. The *Spirulina* samples were dried and used to determine the carbohydrates.

Determining the carbohydrates in *Spirulina* was done by HPLC following the procedure described in the ASTM E1758-01 norm (2003) [18]. A mass of 0.30 g of *Spirulina*, free from extractives, was weighed. Afterwards, 3.00 mL of sulfuric acid (72 wt%) was added under stirring at room temperature for 60 min. After this, the material was transferred to a 250 mL Erlenmeyer flask with 84 mL of water. Therefore, the sulfuric acid was diluted from 72 wt% to 4 wt%. The material was autoclaved at 121 °C for 30 min. The material was filtered and the pH was adjusted to 5-6 by adding calcium carbonate; filtered and analyzed by HPLC, using chromatography equipment, Varian 356-LC (Agilent, USA) with a refractive index (RI) detector at 410 nm in ion exchange Aminex (Bio-Rad, USA) HPX-87H columns (5 mM H_2SO_4 mobile phase, at 50 °C, 0.6 mL/min). The chromatograms were analyzed and quantified by the calibration curve determined for each carbohydrate standard (cellobiose, glucose, xylose, arabinose, manose, galacturonic acid, glucuronic acid and galactose).

The determination of *Spirulina* ash was carried out according to the NREL/TP 510-42622 standard for biomass [19]. Two hundred milligrams (200 mg) of *Spirulina* was put in a pre-weighed crucible and placed in a muffle furnace. The moisture content of the sample was determined using moisture weighing scale (Marte), model ID50, operating at 105 °C to constant weight, with samples of 1.0 g. The measurements were taken in quintuplicate. Nitrogen content was determined using elemental analysis (CHN) equipment from Perkin Elmer, model 2400 with Data Manager software in 2400, operated at 925 °C and 640 °C in combustion furnaces and reduction, respectively, under argon atmosphere. Afterwards, 5 mg sample of *Spirulina* was weighed and analyzed for the composition of C, N and H. The percentage of protein present in the material was calculated by multiplying the nitrogen percentage by 6.25 as described in the NREL/TP-510-42625 standard [20]. The amino acid profile of the *Spirulina* powder was investigated by the Institute of Food Technology (ITAL) in Campinas, Brazil according to White *et al.* [21].

Concerning solubility, 5% (*m*/*v*) of powered *Spirulina* was suspended and homogenized for 30 min in 100 mM of sodium phosphate buffer of pH values varying from four to nine. The suspension was filtered through paper filters of porosity 25 μm. Insoluble fraction was determined after drying the pre-weighted filters.

In order to evaluate the thermal stability of *Spirulina*, thermogravimetric analysis (TGA)/derivative thermogravimetry (DTG) was performed using equipment from TA Instruments Q500. Assays (TG/DTG) were conducted under the following test conditions: heating rate of 10 °C/min in an inert atmosphere (nitrogen) and oxidative atmosphere (synthetic air), with flow rate of 60 mL/min, from room temperature to 800 °C. Approximately 10 mg of each sample was used. Differential scanning calorimetry (DSC) was performed using a TA Instrument model Q100 DSC, with a scan rate of 10 °C/min, and a temperature range from -50 °C to 250 °C. All measurements were conducted under nitrogen atmosphere, using sample mass of about 4 mg.

Infrared spectroscopy was performed in order to characterize the chemical groups present in *Spirulina*. A spectrophotometer Perkin Elmer Spectrum, model 1000, was used, with a wavelength of 400-4,000 cm^{-1}. The samples were pressed into KBr pellets (1 mg Spirulin:100 mg KBr). A plain KBr pellet was used as blank. Crystalline phases present in Spirulin were investigated by X-ray diffraction (XRD) using an X-ray diffractometer Shimadzu XRD-6000, operating at 30 kV and 30 mA with CuKa radiation of 0.154 nm. Assays were performed at room temperature and at angles 2θ between 5° and 40° (0.5°/min). The morphology and uniformity of the commercial Spirulin powder were investigated by electron microscopy. After sample preparation they were coated by the gold deposition method "Sputtering", using Leica equipment MS SCD 050 Sputter Coater (Germany). The micrographs were obtained in an SEM Jeol, JSM 6510 model operating at 20 kV in the form of secondary electrons image (SEI).

EDX is an analytical technique used for elemental analysis of a sample. This analysis was carried out using the same microscope scan of the previous analysis, but was operated in EDX mode (X-rays) at 10 kV. *Spirulina* powder with concentrations of 0.5%, 1.0%, 1.5% and 5% (*m*/*v*) was suspended in 100 mM sodium phosphate buffer pH 7 and homogenized in an ultrasound bath for 15 min. After this, suspension was filtered using filter paper of porosity 25 μm, and absorption spectra were read by UV spectrophotometer Shimadzu UV-Vis 160-IPC in quartz cuvettes and 1 cm optical path. This was done to evaluate the solubility of the alga. Elements Al, Zn and Cu were investigated using this technique, due to its importance in the human diet. For the Flame of the AAS, a PinAACle 900T Perkin Elmer spectrophotometer was used. Digestion of samples was carried out using nitric acid and hydrogen peroxide at 180 °C according to the EPA 3052 norm [22].

3. Results and Discussion

3.1 Chemical Characterization

Spirulina powder showed 10.1% ± 0.05 of moisture content (mean of five measurements). Chemical composition percentages are listed in Table 1. Results for proteins, the most important compound, showed more than 50% in dry basis, which is compatible with the literature references [3, 12].

HPLC chromatographic analysis showed the following carbohydrates for *Spirulina*: cellobiose, 3.2%; glucuronic acid, 8.1%; galacturonic acid, 2.9%; glucose, 8.1%; xylose, 7.2%; arabinose, 3.0%. This means that *Spirulina* contains 11.3% cellulose, 19.3% hemicelluloses and 2.9% pectin in dry basis. These results are in agreement with the observations made by Domozych *et al.* [23] that the *Spirulina* composition is mostly hemicellulosic and less cellulosic. However, the carbohydrate content is higher than that mentioned by

Table 1 Chemical composition of *Spirulina*.

Compound	Amount (% dry weight)	Standard deviation (%)
Protein	53.12	0.12
Carbohydrate	33.6	1.9
Lipids	2.87	0.16
Chlorophyll	0.74	0.02
Ash	9.86	0.35
Total	100.2	

Table 2 Amino acid distribution of the Brazilian *Spirulina* in comparison with other authors.

	Current study	Ref. [26]	Ref. [27]	**
Amino acid	g/100 g protein			
Aspartic acid	8.84	3.90	11.27	-
Glutamic acid	16.07	9.48	9.83	-
Serine	5.18	5.01	4.87	-
Glycine	5.78	2.98	5.45	-
Hystidine*	1.65	10.43	2.10	1.5
Arginine	8.38	8.00	6.98	-
Threonine*	5.72	5.31	5.92	2.3
Alanine	8.32	9.29	9.08	-
Proline	4.17	3.76	4.01	-
Valine*	6.14	6.54	6.78	3.9
Methyonine*	1.93	2.16	2.38	1.6
Cystine*	0.20	0.57	0.86	0.6
Isoleucine*	5.36	6.51	6.40	3.0
Leucine*	9.06	10.18	9.36	5.9
(Phenylalanine + Tyrosine)*	8.78	10.88	10.11	3.8
Lysine	4.43	4.99	4.59	4.5

* indispensible amino acids; ** amino acid intake recommendation (g/100 g protein) for an adult.

Table 3 Quantitative analysis of Zn, Cu and Al by atomic absorption spectrometry (AAS).

Element	Quantity (ppm)	Standard deviation (%)
Zn	2.700	0.700
Cu	0.220	0.050
Al	0.014	0.004

some authors Alvarenga *et al*. [6] and Domozych *et al.* [23] and according to Zeng and Vonshak [24], this can indicate stress due to high salinity of cultivation medium.

Amino acid determination of the *Spirulina* showed a high quality balance of the amino acid composition, therefore it encounters all the indispensible amino acid and at an amount able to supply up to 100% of an adult needs according to FAO/WHO recommendations for protein intake (g/100 g of protein ingestion), with the exception of cystine [25]. The results are presented in Table 2 compared to other authors. The amino acid composition differs greatly among publications, demonstrating that there is a large variability of the algae composition, which depends mostly on its cultivation conditions [26].

Spirulina contains mostly organic elements C, N and O. Other elements, such as S and P are present in proteins and cells. Mg, K, Na and Cl are among the minerals and trace elements essential for human ingestion. Cu, Zn and Al were also detected by qualitative analysis of EDX, and depending on their daily intake, these elements are considered toxic [27]. According to FAO, the maximum daily intake of Al is 7 mg/kg of body weight [28], and according to the US Food and Nutrition Board from the Institute of Medicine of the National Academies [29], the maximum intake for a male adult is 10 mg and 40 mg per day of Cu and Zn, respectively. Flame AAS was also accomplished to quantify these elements as EDX is a qualitative technique that identifies chemical elements of the material, but is not a quantitative characterization method. The results of AAS are shown in Table 3.

Spirulina can concentrate ions found in its growing environment (water and soil). It grows preferably in alkaline water, incorporating minerals and chemical elements. For this reason, the high biosorption capacity of *Spirulina* should be taken into consideration when choosing the culture medium, as it can also absorb heavy metals such as Pb and Hg, if present in the culture medium [9]. The presence of elements, such as Zn, Cu and Al in *Spirulina* does not hinder its use as a diet supplement, as the daily intakes are respected.

Results of the solubility tests showed that the soluble phase for 5% (*m*/*v*) of powered *Spirulina* in water corresponds to about 75 wt % in pH values from six to nine and at pH 4. In pH 5, which corresponds to the isoelectric point of Phycocyanin, only 60 wt% is soluble [30].

The thermal gravimetric assay in both inert and oxidative atmospheres showed a good thermal stability of *Spirulina* at up to 200 °C, as demonstrated in Fig. 2.

3.2 Thermal Characterization

In synthetic air, degradation started at 200 °C and occurred between 200 °C and 380 °C, with the degradation of carbohydrates and protein, and a maximum at 275 °C. Preceding this, there is mass loss corresponding to moisture and volatiles. From 380 °C to 500 °C, oxidation of degraded components occurs. In inert atmosphere, degradation takes place in only one step, after volatile loss, from 200 °C to 500 °C, with a maximum at 285 °C. The presence of a high percentage of protein in the *Spirulina* composition could have led to the formation of char or tar containing nitrogen in the composition, in addition to NO_x gases. According to Becidan *et al.* [31] and Basiuk and Douda [32], linear amides produced from the protein degradation can induce the formation of double bounds between carbon and nitrogen sources in the material. Simkovic and Csomorová [33] also report the formation of heterocyclic compounds between hemicellulose and pectin chains, linked to proteins, this being more resistant to thermal degradation when studying different sources of biomass. The distribution among different N-derivatives after pyrolysis depends on the biomass composition, as well on the reaction speed and final temperature [31, 32]. In the absence of oxygen, the *Spirulina* final residue corresponds to 30% of initial mass, higher than by oxidative conditions (10%). Overall, these results show that *Spirulina* has thermal stability characteristics compatible to be applied in the area of material engineering, food packaging and drug delivery systems and could be explored for the production of biobuels by controlled pyrolysis. Table 4 shows mass content at final degradation steps and corresponding temperatures.

DSC curve shown in Fig. 3 reveals that there is one endothermic event with a minimum at 88 °C, starting at 45 °C and ending at 150 °C that can be associated to protein degradation. This endothermic event suggests that algae processing at temperatures up to 45 °C can be used without thermal degradation of protein.

3.3 Infrared Spectroscopy

Absorption bands related to proteins, fatty acids and phospholipids are present on the *Spirulina* infrared spectrum [9]. The spectrum is shown in Fig. 4. The main peaks identified were: -OH stretching and -NH stretching for glucose and protein (3,500-3,000 cm^{-1}); -CH stretching at 2,925 cm^{-1} and at 2,872 cm^{-1}; at 1,440 cm^{-1} for symmetric bending of $-CH_3$ of acetyl moiety; at 1,237 cm^{-1} for stretching of P=O in phospholipids and nucleid acids; at 1,040, 1,076 and 1,150 cm^{-1} for -CN stretching of proteins; 610 cm^{-1} for stretching and bending of phosphate. Intense peaks at 1,655 cm^{-1} and at 1,543 cm^{-1} indicate the protein specific amide I band (C=O stretching) and amide II related to the Phycocyanin [34].

3.4 Structural Characterization

X-ray diffractometry of *Spirulina* was carried out to investigate the presence of any crystalline structure on the crude powder of the material. The diffractogram (Fig. 5) shows a major amorphous prominence with a maximum 2θ of 20.5°. This amorphous structure is related to the higher content of hemicellulose than cellulose in the carbohydrates composition of this

Table 4 Degradation steps and mass at final point.

Temperature (°C)	Degradation step	Mass (%) Oxidative atmosphere	Mass (%) Inert atmosphere
30-200	Volatile and moisture loss	88	90
200-380	Carbohydrate and protein degradation	50	30
380-500	Oxidation of degraded components	10	30

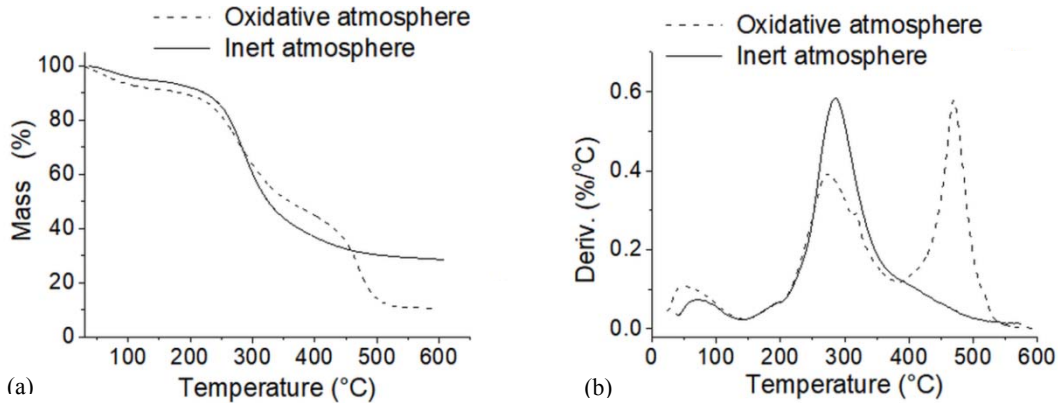

Fig. 2 (a) Thermogravimetric analysis (TGA) and derivative thermogravimetric (DTG) (b) curves of *S. platensis*.

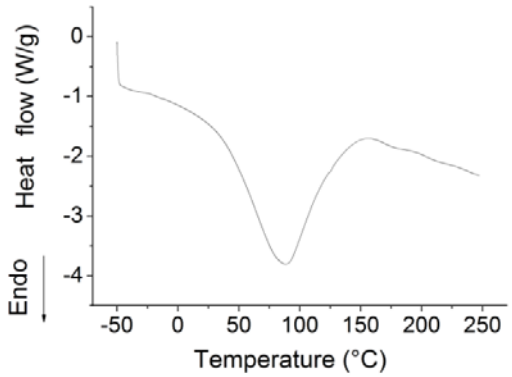

Fig. 3 Differential scanning calorimetry (DSC) curve of *S. platensis*.

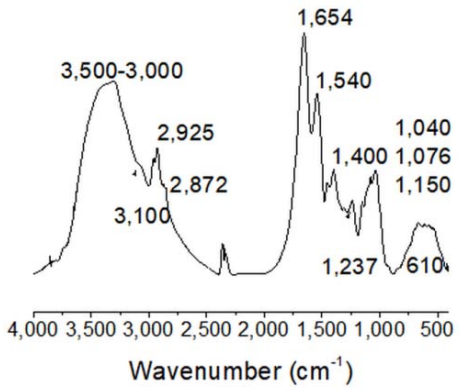

Fig. 4 Fourier transform infrared (FTIR) spectrum of *S. platensis*.

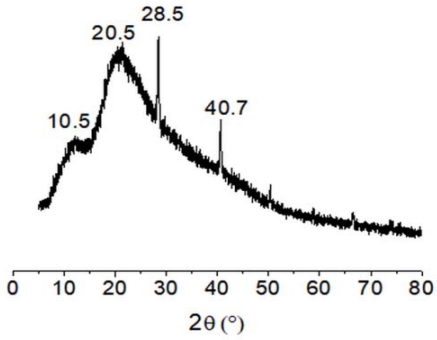

Fig. 5 Diffractogram pattern of *S. platensis*.

blue-green algae [23]. A small ridge is found at 2θ of 10.5°, which can be related to crystalline protein formations present in *Spirulina*. There are additionally two defined prominent peaks at 2θ of 28.5° and of 40.7°. These two peaks are related to KCl [35], a very common supplement used as source of K in the culture medium for algae cultivation [3, 36].

The absorption spectra of solutions with increasing concentrations of *Spirulina* are shown in Fig. 6. The strong absorption at 620 nm is related to the blue color of soluble proteins which are subunits of the blue protein Phycocyanin. Silveira *et al*. [5] demonstrated that phosphate buffer at pH 7 is an optimal solvent for Phycocyanin. Besides the characteristic band of Phycocyanin at 620 nm, strong absorbance bands below 300 nm confirm the presence of many other cellular proteins [30, 37]. The band at 330 nm could indicate the presence of mycosporine-like amino acids (MAAs). MAAs are water-soluble pigments that absorb ultraviolet (UV) radiation at 280-340 nm and have been identified in a number of taxonomically diverse organisms such as fungi, marine heterotrophic bacteria, cyanobacteria, eukaryotic algae, marine invertebrates, fish and a wide variety of other marine organisms in cyanobacteria, the function of MAAs is mainly to protect the cells against solar radiation [38].

Typical SEM micrographs of the microalga are shown in Fig. 7, magnified 5,000 times. From the SEM study, it was observed that during industrial processing and drying to obtain the *Spirulina* powder,

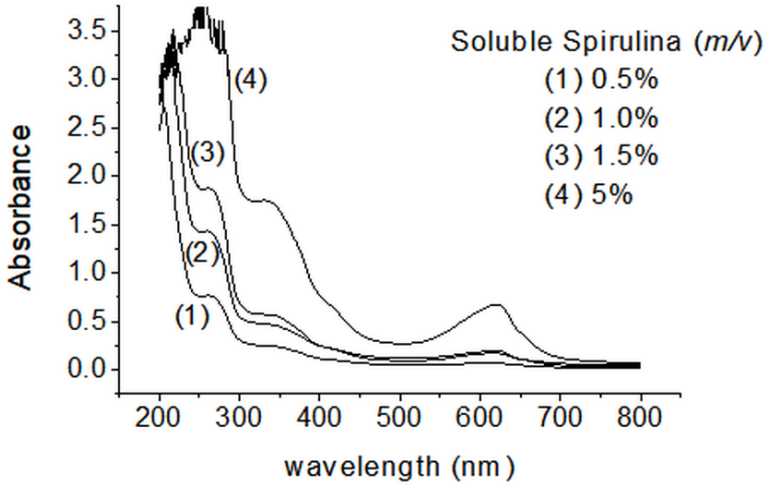

Fig. 6 Ultraviolet-visible (UV-vis) spectra of *Spirulina* solutions in water.

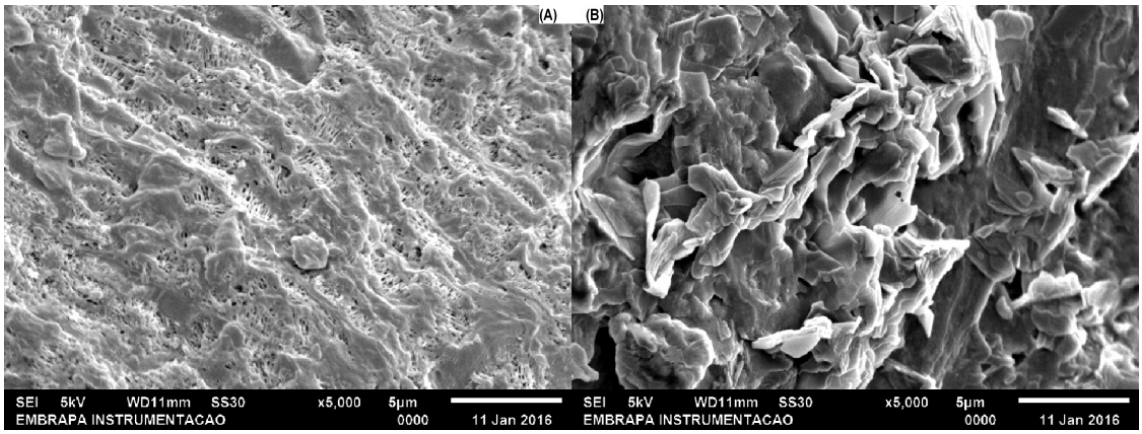

Fig. 7 Scanning electron microscopy (SEM) images of *Spirulina* powder, magnified 5,000 times.

the *Spirulina* filaments were broken into particles of diameter ranging from 10 µm to 200 µm. Two different morphologies were found for these *Spirulina* particles. The first one was homogeneous and soft and the other rougher and more heterogeneous (Figs. 7A and 7B, respectively).

4. Conclusions

Commercial *S. platensis* grown in Brazil was characterized in this work. Brazilian *Spirulina* has 53% protein, 33% carbohydrates, 3% lipids and 10% ash: a chemical composition very favorable to be use in food besides animal feed and in other applications such as producing biodegradable films. The X-ray diffractogram for *Spirulina* confirms the main amorphous characteristic of the algae, although identifying a very intense crystalline peak due to the presence of minerals (KCl) added in the culture medium. *Spirulina* also has good thermal properties, and is stable up to 200 °C, which shows that it is suitable to be used in biocomposites. Additionally, due to high mass residue generated after pyrolysis, *Spirulina* could be explored for the biofuel production. Phycocyanin was confirmed in the *Spirulina* and was identified by the characteristic peaks in the FTIR spectrum, by UV-vis spectroscopy, where an intense peak at 620 nm was visible and the decrease in the solubility of *Spirulina* at pH 5, the isoelectric point of Phycocyanin. In sum, the *Spirulina* represents a valuable and multifold renewable material which can provide exceptional properties for various applications.

Acknowledgments

The authors would like to thank SisNANO/MCTIC, Capes, CNPq, FAPEMIG, FINEP and Nanotechnology Network for Research in Agriculture, Rede AgroNano, Embrapa.

References

[1] Soni, R. A., Sudhakar, K., and Rana, R. S. 2017. "*Spirulina*—From Growth to Nutritional Product: A Review." *Trends in Food Science & Technology* 69: 157-71.

[2] Sili, C., Torzillo, G., and Vonshak, A. 2012. "Arthrospira (*Spirulina*)." *Ecology of Cyanobacteria* 2: 677-705.

[3] Habib, M. A. B., Parvin, M., Huntington, T. C., and Hasan, M. R. 2008. "A Review on Culture, Production and Use of *Spirulina* as Food for Humans and Feeds for Domestic Animals and Fish." In *FAO Fisheries and Aquaculture Circular No. 1034.* Rome: Food and Agriculture Organization of the United Nations.

[4] Carvalho, L. F. D., Moreira, J. B., Oliveira, M. S., and Costa, J. A. V. 2018. "Novel Food Supplements Formulated with *Spirulina* to Meet Athletes' Needs." *Brazilian Archives of Biology and Technology* 61: 1-11.

[5] Silveira, S. T., Burkert, J. F. M., Costa, J. A. V., Burkert, C. A. V., and Kalil, S. J. 2007. "Optimization of Phycocyanin Extraction from *Spirulina platensis* Using Factorial Design." *Bioresource Technology* 98: 1629-34.

[6] Alvarenga, R. R., Rodrigues, P. B., Cantarelli, V. S., Zangeronimo, M. Z., Silva Jr., J. W., Silva, L. R., Santos, L. M., and Pereira, L. J. 2011. "Energy Values and Chemical Composition of Spirulina (*Spirulina platensis*) Evaluated with Broilers." *Revista Brasileira de Zootecnia* 40 (5): 992-6.

[7] Reichert, C. C., Reinehr, C. O., and Costa, J. A. V. 2006. "Semicontinuous Cultivation of the Cyanobacterium *Spirulina platensis* in a Closed Photobioreactor." *Brazilian Journal of Chemical Engineering* 23 (1): 23-8.

[8] Chaiwong, K., Kiatsiriroat, T., Vorayos, N., and Thararax, C. 2013. "Study of Bio-Oil and Bio-Char Production from Algae by Slow Pyrolysis." *Biomass and Bioenergy* 56: 600-6.

[9] Doshi, H., Ray, A., and Kothari, I. L. 2007. "Biosorption of Cadmium by Live and Dead Spirulina: Kinetics and SEM Studies." *Current Microbiology* 45: 213-8.

[10] Moraes, C. C., Sala, L., Cerveira, G. P., and Kalil, S. J. 2011. "C-Phycocyanin Extraction from *Spirulina platensis* Wet Biomass." *Brazilian Journal of Chemical Engineering* 28 (1): 45-9.

[11] Iannace, S., Nocilla, G., and Nicolais, L. 1999. "Biocomposites Based on Sea Algae Fibers and Biodegradable Thermoplastic Matrices." *Journal of Applied Polymer Science* 73: 583-92.

[12] Zeller, M. A., Hunt, R., Jones, A., and Sharma, S. 2013. "Bioplastic and Their Thermoplastic Blends from Spirulina and Chlorella Microalgae." *Journal of Applied Polymer Science* 130 (5): 3264-75.

[13] Ortega-Calvo, J. J., Mazuelos, C., Hermosin, B., and Saiz-Jimenez, C. 1993. "Chemical Composition of Spirulina and Eukaryotic Algae Food Products Marketed in Spain." *Journal of Applied Phycology* 5: 425-35.

[14] Folch, J., Lees, M., and Sloane Stanley, G. H. 1957. "A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues." *Journal of Biological Chemistry* 226: 497-509.

[15] Pohndorf, R. S., Camara, A. S., Larrosa, A. P. Q., Pinheiro, C. P., Strieder, M. M., and Pinto, L. A. A. 2016. "Production of Lipids from Microalgae *Spirulina* sp. Influence of Drying, Cell Disruption and Extraction Methods." *Biomass and Bioenergy* 93: 25-32.

[16] Akoh, C. C. E., and Min, D. B. 2017. *Food Lipids: Chemistry, Nutrition, and Biotechnology.* Florida: CRC Press.

[17] Kobayashi, Y., Kurimura, Y., Sakamoto, E., and Tsuji, Y. 1997. "Selective Extraction of Astaxanthin and Chlorophyll from the Green Alga *Haematococcus pluvialis* M." *Biotechnology Technology* 11 (9): 657-60.

[18] ASTM E1758-01. 2003. "Standard Test Method for Determination of Carbohydrates in Biomass by High Performance Liquid Chromatography." In *Annual Book of ASTM Standards*, Vol. 11.05. West Conshohocken, PA: American Society for Testing and Materials.

[19] Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., and Templeton, D. 2008. "Determination of Ash in Biomass." NREL/TP-510-42622, National Renewable Energy Laboratory, Golden, Co.

[20] Hames, B., Scarlata, C., and Sluiter, A. 2008. "Determination of Protein Content in Biomass." NREL/TP-510-42625, National Renewable Energy Laboratory, Golden, Co.

[21] White, J. A., Hart, R. J., and Kry, J. C. 1986. "An Evaluation of the Waters Pico-Tag System for the Amino Acid Analysis of Food Materials." *Journal of Automatic Chemistry* 8: 170-7.

[22] Environmental Protection Agency. 1996. "Method 3052: Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices." In *Test Methods for Evaluating Solid Waste*. Washington, DC: US Environmental Protection Agency.

[23] Domozych, D. S., Stewart, K. D., and Mattox, K. R. 1980. "The Comparative Aspects of Cell Wall Chemistry in the Green Algae (Chlorophyta)." *Journal of Molecular Evolution* 15: 1-12.

[24] Zeng, M. T., and Vonshak, A. 1998. "Adaptation of *Spirulina platensis* to Salinity-Stress." *Comprehensive Biochemistry Physics A* 120: 113-8.

[25] WHO. 2002. *FAO/WHO/UNU Joint Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition.* Geneva, April 9-16, 2002. http://whqlibdoc.who.int/trs/WHO_TRS_935_eng.pdf.

[26] Volkmann, H., Imianovsky, U., Oliveira, J. L. B., and Sant'Anna, E. S. 2008. "Cultivation of *Arthrospira* (*Spirulina*) *platensis* in Desalinator Wastewater and Salinated Synthetic Medium: Protein Content and Amino-Acid Profile." *Brazilian Journal of Microbiology* 39: 98-101.

[27] Becker, E. W. 2007. "Micro-algae as a Source of Protein." *Biotechnology Advances* 25: 207-10.

[28] WHO. 1989. *Evaluation of Certain Food Additives and Contaminants.* Geneva, Switzerland. http://whqlibdoc.who.int/trs/WHO_TRS_776.pdf.

[29] IOM. 2001. *Recommended Dietary Allowance and Adequate Intake Values, Vitamins and Elements.* Washington, DC: Food and Nutrition Board of the National Academies Institute of Medicine. https://ods.od.nih.gov/HealthInformation/Dietary_Reference_Intakes.aspx.

[30] Kao, O. H. W., Edwards, M. R., and Berns, D. S. 1975. "Physical-Chemical Properties of C-Phycocyanin Isolated from an Acido-Thermophilic Eukaryote, *Cyanidium caldarium.*" *Biochemistry Journal* 147: 63-70.

[31] Becidan, M., Skreiberg, O., and Hustad, J. E. 2007. "NO_x and N_2O Precursors (NH_3 and HCN) in Pyrolysis of Biomass Residues." *Energy & Fuels* 21: 1173-80.

[32] Basiuk, V. A., and Douda, J. 2000. "Pyrolysis of Poly-Glycine and Poly-L-Alanine: Analysis of Less-Volatile Products by Gas Chromatography/Fourier Transform Infrared Spectroscopy/Mass Spectrometry." *Journal of Analytical and Applied Pyrolysis* 55: 235-46.

[33] Simkovic, I., and Csomorová, K. 2006. "Thermogravimetric Analysis of Agricultural Residues: Oxygen Effect and Environmental Impact." *Journal of Applied Polymer Science* 100: 1318-22.

[34] JCPDS-ICDD Database, Ref. No. 01.073.0380. International Centre of Diffraction Data, ICDD, USA. 2004.

[35] Wang, C.-Y., Fu, C.-C., and Liu, Y.-C. 2007. "Effects of Using Light-Emitting Diodes on the Cultivation of *Spirulina platensis.*" *Biochemical Engineering Journal* 37: 21-5.

[36] Chen, T., Wong, Y.-S., and Zheng, W. 2006. "Purification and Characterization of Selenium-Containing Phycocyanin from Selenium-Enriched *Spirulina platensis*." *Phytochemistry* 67: 2424-30.

[37] Sinha, R. P., Klisch, M., and Häder, D.-P. 1999. "Induction of a Mycosporine-Like Amino Acid (MAA) in the Rice-Field Cyanobacterium *Anabaena* sp. by UV Irradiation." *Journal of Photochemistry and Photobiology B* 52: 59-64.

[38] Matsui, K., Nazifi, E., Kunita, S., Wada, N., Matsugo, S., and Sakamoto, T. G. 2011. "Novel Glycosylated Mycosporine-Like Amino Acids with Radical Scavenging Activity from the Cyanobacterium *Nostoc commune*." *Journal of Photochemistry and Photobiology B* 105: 81-9.