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Spirulina platensis biomass cultivated in Southern Brazil as a source of essential minerals and other nutrients

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The aim of this study was to determine elemental constituent of microalgal biomass *Spirulina platensis* LEB-18 grown on the shores of Mangueira Lagoon, South of Brazil. The spread of inoculum was performed in standard Zarrouk medium. When the microalgae concentration reached 0.50 g L^{-1}, the biomass was separated by filtration and dried in a tray dryer at 50°C for 5 h. The harvested biomass was then ground in a ball mill, sieved to achieve a particle size of 88 µm (~ 300 mg) in triplicate, digested with concentrated HNO_3 and diluted with up to 5% HNO_3 for subsequent quantification. Mineral content was determined by inductively coupled plasma optical emission spectrometry with axial view and other nutrients by standard methods. Among the macro minerals, in µg g^{-1} ± standard deviation, calcium (15.108±529), sodium (14.6552±485) and potassium (14.036±109) were found in the largest amount. The micro mineral present in greater quantity, in µg g^{-1} ± standard deviation, was the iron (956±9) and nutrients present in the following order: protein (58.5%), ash (12.0%), carbohydrate (7.5%), lipid (7.0%) and crude fiber (0.95%). Results were compared with the values of minerals for human consumption recommended by the Food and Drug Administration and also with the Recommended Daily Intake (RDI) of minerals by Brazilian Legislation. The findings proved promising for the use of *S. platensis* strain LEB-18 as an alternative source of essential minerals and other nutrients for the human body.

Key words: Cyanobacterium, chemical element, nutritional importance, optical emission spectrometry.

INTRODUCTION

The use of single-cell protein which refers to the dried cells of microorganisms like yeast, bacteria and microalgae has been present for thousands of years in the world population, mainly in the form of drugs. In recent decades, biochemical engineering has been devoted to developing new methods for food processing. The cyanobacteria currently cultivated in large scale systems are economically viable sources of protein in food because they often meet the requirements of this nutrient in the diet and, moreover, through them you can get other human consumer products (Zepka et al., 2010).

Cyanobacteria as a source of single-cell protein have certain advantages over the use of other microorganisms because of its rapid growth and quantity and quality of protein. Among the microalgae, the genus *Spirulina* contains about 60 to 70% of proteins, nucleic acids and amino acids recommended by the Food and Agriculture Organization. It also contains beta-carotene, absorbable iron and other minerals, as well as high levels of vitamins, phenolic compounds, gamma-linolenic acid and other

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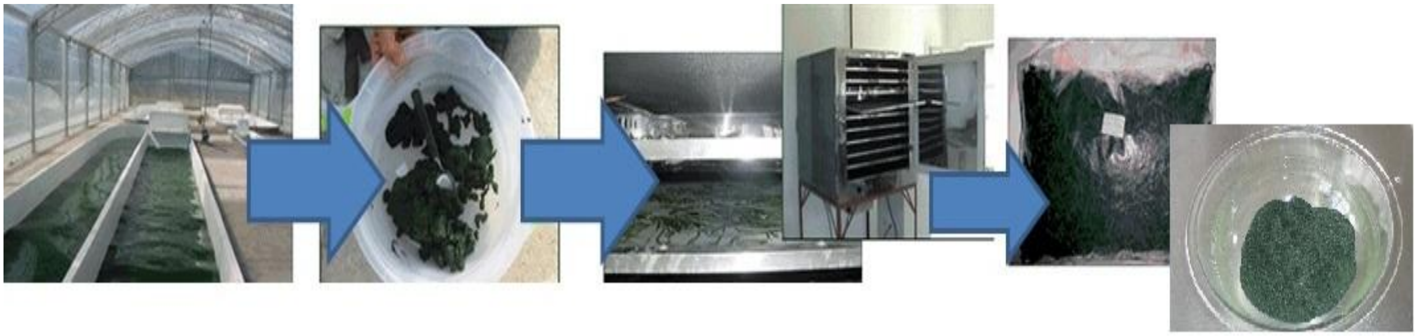

Figure 1. Biomass processing- left to right: cultivation, filtration, drying and storage.

Table 1. Heating program of the microwave oven for the sample digestion.

Stage	Ramp (min)	Temperature (°C)	Residence time (min)	Pressure (bar)
1	20	170	10	35
2	5	200	25	35
3	5	50	20	35

essential fatty acids (Belay et al., 1993).

Environmental factors affect cyanobacteria growth due to their physiological requirements. It is important to determine the optimum culture conditions for the achievement of high yields of microalgae in standard media. Researchers have been devoted to optimizing the cultivation of Spirulina *platensis* (Çelekli and Yavuzatmaca, 2009; Çelekli et al., 2009; Teimouri et al., 2013).

Spirulina is marketed and consumed in Germany, Brazil, Chile, Spain, France, Canada, Belgium, Egypt, United States, Ireland, Argentina, Philippines, India, Africa and other countries where public administration, sanitary organisms and associations have been approved for human consumption (Henrikson, 1994). Some of the best worldwide known companies producing *Spirulina* are: Earthrise Farms (USA), Cyanotech (USA), Hainan DIC Microalgae Co., Ltd (China), Marugappa Chettir Research Center (India), Genix (Cuba) and Solarium Biotechnology (Chile) (Belay, 1997).

Currently, the Federal University of Rio Grande (FURG) is cultivating the microalgae *Spirulina platensis* for inclusion in human diet, especially strain LEB-18. In terms of nutritional application, this strain has been analyzed and compared with values internationally accepted by Food and Drug Administration (FDA) for their fatty acid profiles (Radmann and Costa, 2008), amino acids profiles and content of heavy metals (Morais et al., 2009).

This study aims to determine the content of some essential minerals (Ca, P, Mg, Na, P, S, Cr, Fe, Zn, Mn, Mb and Cu) and other nutritional components (proteins, ash, carbohydrate, lipid and crude fiber) in *S. platensis* strain LEB-18, and thus contribute to its possible application in food in order to combat nutritional deficiencies.

MATERIALS AND METHODS

Obtaining and preparing the microalgal biomass

Used *S. platensis* strain LEB-18 was cultivated, produced by the Federal University of Rio Grande, Brazil (Morais et al., 2008). The pilot plant for production of biomass was located near the shore of Mangueira Lagoon (33° 30′ 13″ S; 53° 08′ 59″ W) and consisted of raceway tanks of different dimensions and volumes depending on their purpose. The tanks are protected from UV radiation by the use of a transparent film. When the concentration reached 0.50 g L^{-1} (maximum growth), the biomass was separated by filtration, washed and dried at 50°C for 5 h and stored under refrigeration (Figure 1). *Spirulina* biomass value was calculated through Optical Density (OD) measurements using a spectrophotometer (UV/VIS to 670 nm) and a calibration curve of OD against dry weight (g L^{-1}) of *Spirulina* biomass. After harvesting, the biomass was crushed into a ball mill (88 µm) for analyses.

Sample preparation and determination of minerals

Samples (~300 mg) were weighed in triplicate and digestion was made in a microwave oven (Speedwave four model, Berghof, Germany). The heating program is described in Table 1. For digestion, 5 mL of HNO_3 concentrated and redistilled (Merck) and 0.5 mL of HF 40% (Merck) were used. After digestion, samples were diluted 5 to 500 times in 5% HNO_3 for subsequent quantification by inductively coupled plasma optical emission spectrometry (ICP OES).

The determinations were made in an ICP OES Perkin Elmer (model Optima 4300 DV, Shelton, USA) with axial view. The introduction of the samples in the spectrometer was done using a

Table 2. Operating conditions of the equipment ICP OES*.

Parameter	Condition
Radiofrequency power (W)	1400
Principal gas flow rate (L min^{-1})	15
Auxiliary gas flow rate (L min^{-1})	0.2
Nebulizer gas flow rate (L min^{-1})	0.7
	Ca 315.887
	Fe 238.204
	K 766.490
	Mg 279.077
	Na 588.995
Wavelength (nm)	P 214.914
	S 181.975
	Cr 267.717
	Cu 324.759
	Mn 259.374
	Zn 213.857

*Inductively coupled plasma optical emission spectrometry.

Table 3. Mineral contents of *S. platensis* LEB-18 (μg g^{-1} ± SD) compared with mineral values presented by the Food and Drug Administration (FDA) in *Spirulina* for human consumption (μg g^{-1}) and the recommended daily intake (RDI) of minerals for children and adults (μg).

Mineral	*S. platensis* LEB-18		FDA	RDI for children*	RDI for adults
	Mean	SEM			
Macro and micro minerals					
Na	19.5	99	14.0	-	$5x10^5$
Ca	15.1	529	4.6	$5x10^5$ - $7x10^5$	$1x10^6$
K	14.0	109	18.6	-	2x106
P	8.7	19	10.0	$46x10^4$ - $125x10^4$	$7x10^5$
Mg	4.7	83	7.6	$6x10^4$ - $10x10^4$	26 $x10^4$
S	6.7	323	-	-	-
Fe	956	9	533	$6x10^3$ - $9x10^3$	$14x10^3$
Mn	106	2	32	$12x10^2$ - $15x10^2$	$23x10^2$
Zn	35.4	0.8	27	$41x10^2$ - 56 $x10^2$	$7x10^3$
Cu	11.9	0.2	7	340 - 440	$9x10^2$
Cr	4.0	0.2	-	11 - 15	35

*Children 1 to 10 years old.

GemCone nebulizer and a cyclonic spray chamber. The other conditions of the equipment are described in Table 2. The plasma was formed from argon (White Martins, São Paulo, Brazil) with a purity of 99.996% (AOAC, 2000).

Proximate analyses

The microalgal biomass was analyzed by following methods described by Association of Official Analytical Chemists (AOAC, 2000). Total lipids were determined on biomass and diets by the method of Bligh and Dyer (1959).

RESULTS AND DISCUSSION

As shown in Table 3, the analyzed macro minerals were present in *S. platensis* LEB-18 biomass in the following order: calcium, sodium, potassium, phosphorus, sulfur and magnesium. Among the micro minerals, iron was found in the largest amount, followed by manganese, zinc, copper and chromium, respectively. For sample analysis, this study compared it with the data recommended by the Food and Drug Administration (FDA, 2013), which sets nutrient levels for *S. platensis* or *Spirulina* maxima to be suitable for human consumption, as well as the Recommended Daily Intake (RDI) established by the Brazilian Ministry of Health (Brazil, 2005). In comparison with RDI, the higher recommended value will be used, either for adults or children.

Calcium is an essential mineral that carry out various biological functions. Studies have demonstrated an association between low calcium intake and chronic diseases such as osteoporosis, colon cancer, hypertension and obesity (Pereira et al., 2009). However, most of the Brazilian population has intakes of calcium below recommended levels (1 x 10^6 µg per day for an adult individual) (Brazil, 2005). The study sample, *S. platensis* LEB-18, had 15.108 µg g^{-1}, and 66 g of biomass would supply daily calcium requirement.

The sample studied by our research group showed 14.6552 µg g^{-1} sodium, a value close to that established by the FDA for *S. platensis*. However, when compared with RDI (Brazil, 2005), 157 g of the biomass is estimated to contain the 2.3 x 10^5 g of sodium envisaged. Sodium, normally found in foods in the form of salt (sodium chloride), is also an essential mineral for several vital functions: muscle contraction, maintenance of blood pressure, nerve transmission and fluid balance. Sodium chloride or salt or the "white gold" is associated with health and healthy, "*salus*" and "*salubris*", derived from Latin "salt". However, nowadays it is known that the high consumption of this mineral may trigger various body disorders. Evidence of the association between high sodium intake and cardiovascular disease and stroke have been observed apart from association with increased blood pressure.

Functions of potassium include maintenance of intracellular fluid, muscle contraction, conduction of stimuli, heart rate control, energy production and synthesis of proteins and nucleic acids. An adult individual considered healthy requires 2 x 10^6 g of potassium per day to carry out the activities mentioned above, which could be achieved with the consumption of 143 g of *S. platensis* LEB-18.

As previously mentioned, minerals, phosphorus, sulfur and magnesium are also vital components for animals. Phosphorus, sulfur and magnesium were found in the biomass of *S. platensis* strain LEB-18 as 8.736, 6.764 and 4.722 µg g^{-1} respectively, which corresponds to 1.25, 12.3 and 1.8% of RDI. The amounts of phosphorus and magnesium in the sample are slightly lower than those recommended by the FDA in *Spirulina* for human consumption, but sulfur is not cited by U.S. law, so it is not possible to compare the biomass.

The micronutrients in *S. platensis* LEB-18 biomass showed that, as compared to the data recommended by the FDA, four of them were found in higher amounts, namely iron, manganese, zinc and copper. Chromium content was not cited by the FDA (2013). Iron content is highlighted. Each 1 g of biomass corresponds to 6.8%of RDI for adults and 10.5-16% for children.

Iron is an essential nutrient which is present in many foods. The iron content found in cereal and grains do not differ greatly, with an average of 25-80 µg g^{-1}. Taking as reference the food known worldwide as a source of iron (Braga and Mendonça, 2010), such as raw liver (56 µg Fe g^{-1}), brindle raw beans (18.6 mg Fe g^{-1}), and brown sugar (8.6 µg Fe g^{-1}) (Taco, 2011), *S. platensis* LEB-18 can be considered as a far more representative source of the mineral.

Biologically, manganese is associated with the formation of connective tissues and bones, growth and reproduction, and metabolism of carbohydrates and lipids; zinc, as a structural and/or functional component of several metal-enzymes and metal-proteins, participates in many reactions of cellular metabolism, including physiological processes such as immune function, antioxidant defense, growth and development (Mafra and Cozzolino, 2004; Pedraza et al., 2011); copper can be found in biological tissues in the form of organic complexes such as metal-proteins with enzymatic activity.

The use of oxygen during cell respiration, energy utilization and synthesis of essential compounds are examples of metabolic reactions mediated by enzymes which require the presence of copper to have catalytic activity. Thus, although required at low concentrations, they are essential for human metabolism.

In this study, *S. platensis* strain LEB-18 was also seen as an excellent source of chromium (each 1 g of biomass is capable of supplying 11.5% of RDI). This mineral is important in maintaining glucose metabolism possibly because of the potentiation of insulin action at the cell membrane level, thus influencing the metabolism of carbohydrates, lipids and proteins (Casey and Wabravens, 1988).

Al-Dhabi (2013) stated that minerals of commercial *Spirulina* biomasses found 0.533-6.225, 0.002-0.036 and 0.005-2.2 µg g^{-1} of zinc, magnesium and manganese, respectively. Ramírez-Moreno and Olvera-Ramírez (2006) reported that the nutritional composition of *Spirulina* sp. for human consumption should contain 1-14% potassium, 0.4-0.5% sodium, 0.3-0.7% potassium, 0.1-0.4% calcium, 0.1-0.2% magnesium, 0.03-0.05% iron, 0.005% manganese, 0.003% zinc and 0.0012% copper.

In this study, we found, on a dry basis, 58% protein, 7.5% carbohydrate, 7% fat and 0.5% crude fiber in the biomass of *S. platensis* LEB-18. According to Habib et al. (2008), *S. platensis* is a cyanobacterium with the following chemical composition: proteins (50-70%), carbohydrates (15-25%), lipids (6-8%), ash (7-13%), fiber (8-10%) and moisture content (3-7%). Madkour et al. (2012) found that *Spirulina* biomass grown at different culture conditions consisted of 37.79-52.95% protein, 13.20-24.5% carbohydrate, and 5.64-15.39% lipids. Oliveira et al. (2009) studied the influence of drying

Spirulina and found in dry samples 64.1% protein, 10.3% carbohydrate, 8.6% lipids and 7.3% ash.

Spirulina is valued by the variety of nutrients it contains, some of which are not synthesized by the human body. Due to its richness of micro and macro nutrients, is a complete food both qualitatively and, if consumed in certain doses, quantitatively. Moreover, it is a food with the highest content of different nutrients per weight unit, and 20 g of this cyanobacterium meet all human body needs (Phang et al., 2000).

Taking into account the contents of minerals, proteins, carbohydrates and lipids present in *Spirulina* strain LEB-18, an interesting result of these nutritional components in a single source was observed. The study of Moreira et al. (2013), in which malnourished rats were fed for 30 days with a diet supplemented with *S. platensis* LEB-18, had great findings regarding the hematological and biochemical profile of blood, thus indicating the cyanobacterium studied as an excellent supplement for human consumption.

Conclusion

The cultivation of *S. platensis* LEB-18 in southern Brazil in Zarrouk medium represents an attractive option for the development of nutritional supplementation because of the high content of proteins and minerals, especially iron, present in the cell biomass.

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