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RESEARCH

Effect on protein content and digestibility of traditional bread by incorporating *Arthrospira platensis*

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Abstract

Since the world population is expected to reach 9.8 billion by 2050, a global demand for alternative proteins to traditional ones is expected. Alternative plant-based proteins are experiencing significant global demand as they offer similar characteristics, at a relatively more affordable cost than animal proteins. In recent years, the industrial production of microalgae biomass has received attention attributable to its rich content of quality proteins. On the other hand, due to their low carbon footprint, wastewater treatment benefits and carbon credit from industrial CO_2 conversion, microalgae-based proteins have the potential to provide ecologic benefits. In this sense, the study of new species of protein-rich microalgae is an area of ongoing research. Thus, the main objective of the present study was to culture and characterize microalgal isolates of *Arthrospira platensis* from the Yaragui Lagoon of the Paraguayan Chaco. Nitrogen protein was studied by Kjeldahl method and the same technique was applied to obtain the non-protein nitrogen, but after treatment of the sample with trichloroacetic acid to remove proteins. The hydrosoluble protein content was measured by the Lowry method after freezing and thawing followed by sonication, and was compared with the Kjeldahl method. The nitrogen-to-protein conversion factor for biomass experimentally obtained was 6.2508 for nitrogen measured by Kjeldahl. Protein total content was around 67% and the hydrosoluble fraction corresponds to 66% of it. The proximate composition, the protein profile, the molecular structure and functional groups were also studied. Finally, the addition of *Arthrospira* to bread showed that nutritional quality was improved and the level of protein digestibility achieved was satisfactory. Thus, the results suggest that using *A. platensis* from the Yaragui Lagoon of the Paraguayan Chaco to make rich protein products provides a viable alternative for human consumption.

Keywords Cyanobacteria · Spirulina · Microalgae · Bread · Proteins · Digestibility

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Introduction

Microalgae and cyanobacteria are a source of valuable food and ingredients. They contain proteins, lipids and bioactive compounds with various bioactivities (Lafarga and Acién 2022). The use of microalgae as a source of protein has been highlighted mainly because, unlike traditional terrestrial crops, they differ in their resource requirements as they do not need arable soil or fresh potable water for their sustenance. In addition, microalgae have demonstrated significant growth capabilities in aqueous environments of varying salinity, including seawater and wastewater as growth media (Hashemian et al. 2019; Ahmad et al. 2022). Protein production from microalgae using photobioreactors is generally more efficient than growing plants or raising animals (Ciami et al. 2021). This is not only because of higher photosynthetic efficiency or better utilization of nutrients (nitrogen, phosphorus, etc.), but also because of the potential to control the process and produce food throughout the year. In addition, photobioreactors can be installed on uncultivable land and even in cities.

As the world human population is expected to reach 9.8 billion by 2050 (UN 2023), traditional food systems must change in order to meet the coming food demands. In this context, alternative protein sources acting as meat and dairy substitutes attract global research, development and innovation (i + D&I) interest and investment (FAO 2023; Severo et al. 2024). Significant global changes such as increased urbanization, an aging population, and increased awareness of environmental issues, particularly concerning water footprint, land use, greenhouse gases (Lee et al. 2023), and the health impacts of food consumption, are driving shifts in consumption patterns, in particular an increase in dietary protein intake (Wang et al. 2021). Furthermore, new technologies and innovations in product development, along with changes in dietary patterns, such as the rise in the vegan population, may potentially contribute to reducing the environmental impact of food production (Alcorta et al. 2021; Arwanto et al. 2022; Lee et al. 2023).

Microalgae are considered a food of the future due to their superior nutritional profile, which includes a more complete amino acid profile and several micronutrients. They also contain higher concentrations of vitamins and carotenoids such as vitamin B12, vitamin E, β-carotene, α-carotene, astaxanthin, and lutein (Begum et al. 2016; Henríquez et al. 2016; Sun et al. 2016), compared to conventional sources of typical plant protein (Vrenna et al. 2021). The biomass is mainly composed of protein content at about 40 to 70%, followed by significant amounts of carbohydrates (10 to 25%), lipids (5 to 30%), minerals (5 to 25%), and pigments as a minor component at about 1 to 5% (Deprá et al. 2018). They also contain polyunsaturated fatty acids (PUFAs), including the healthful docosahexaenoic acid (DHA) and saturated fatty acids (SFA) (Sohedein et al. 2020). In addition, the high digestibility of microalgae proteins is another benefit (Lafarga and Acién 2022). The chemical composition and amino acid profile of algae are observed to be highly variable and depend on the species and the environmental conditions in which they grow, including geography, season, water temperature, nutrient content, and life cycle stage (Auoir et al. 2017). In this regard, the study of new protein-rich microalgae species is an ongoing area of research.

Arthrospira platensis has been extensively characterized in the scientific literature, with many examples of its use as a nutritional supplement (Chang and Liu 2024; Pehlivanov et al. 2024; Demarco et al. 2025). However, the particular strain from the Yaragüí Lagoon in the Paraguayan Chaco is littlr studied, much less its growth under controlled conditions to maximize protein yield. The main objective of this study was therefore to cultivate and characterise *A. platensis* isolates from this lagoon and evaluate their potential use as a food additive. The nitrogen-to-protein conversion factor was determined by comparing and correlating the nitrogenous protein, total protein, and water-soluble protein contents. Additionally, the proximate composition, protein profile, molecular structure, and functional groups were studied. Finally, the nutritional effect of adding *A. platensis* to bread was evaluated, with a focus on changes in protein digestibility, offering an alternative application in the food industry for human consumption.

Materials and methods

Samples

The samples were taken from the Yaragui Lagoon, Praguay, located 4 km from Colonia Lolita and 59 km from the Loma Plata turnoff, Transchaco Route; at an altitude of 113 m above mean sea level. It has an approximate extension in one of its branches of 17 ha and an average depth of 1.30 m.

Microalgae culture and medium compositions

Arthrospira platensis was isolated and identified by its morphological characteristics (López et al. 2018; Villalba et al. 2018). The pure *A. platensis* was cultured in Zarrouck's medium consisting of (g L^{-1}): $NaHCO_3$ 16.8; K_2HPO_4, 0.5; $NaNO_3$ 2.5; NaCl 1; $MgSO_4$ 0.2; $CaCl_2.2H_2O$, 0.04; $FeSO_4$ 0.01; EDTA.2Na, 0.08; and 1 mL of solution A5 consisting of (g L^{-1}): H_3BO_3, 2.86; $ZnSO_4.7H_2O$, 0.22; $MnCl_2.4H_2O$, 1.81; $Na_2MoO_4.2H_2O$, 0.39; $CuSO_4.5H_2O$, 0.08.

Fig. 1 *Arthrospira platensis* from Yaragui Lagoon of the Paraguayan Chaco cultivation in photobioreactors

The photobioreactor system consisted of six 25-L plastic bottles illuminated by an external light source of 5 cool-white fluorescent lamps of 36 kW and a total luminous intensity of 12,500 lm (36-W T8 tungsten filament lamps; Philips Co., Poland) (Fig. 1). The inoculum size of 240×10^3 cells mL^{-1}, was inoculated into the photobioreactor, which was operated under a 24-h light photoperiod at 25 °C ± 2 °C and pH 9. A constant aeration of 0.45 vvm, previously filtered with 0.22 µm nylon membrane filters, was applied to a volume of 13 L of culture. During growth, a sample was collected at regular intervals to determine microalgal biomass concentration. Once the cultures reached the stationary growth phase, the entire culture broth was harvested by continuous centrifugation and concentrated to biomass slurry, which was washed with distilled water and centrifuged at 2500 rpm for 10 min. The dry cell weight of the microalgal biomass was obtained by filtering the culture through a cellulose acetate membrane filter (0.45 µm pore size, 25 mm diameter) and then dried drying at 65 °C until the weight was stable.

Biomass composition analysis

Protein content was determined by the Kjeldahl method after acid digestion (AOAC 991.20, 2005) and calculated using a nitrogen-to-protein conversion factor of N × 6.25. To precipitate the biomass microalgae protein, 100 g L^{-1} trichloroacetic acid was added to the sample and allowed to stand for 1 h. Non-protein nitrogen present in the supernatant was then determined by the Kjeldahl method. Protein nitrogen was calculated as the difference between total nitrogen by Kjeldhal and non-protein nitrogen. The Lowry method (Lowry et al. 1951) was used to measure the fraction of water-soluble protein content. The biomass was pretreated according to Tavanandi et al. (2018) with modifications. 10 mL of 0.1 M phosphate buffer (35 mL) pH 6.8 was added to 20 mg of dry biomass and kept at room temperature (27 ± 2 °C) for 4 h with intermittent shaking. The suspension was then subjected to freezing and thawing (4 h freezing and 1 h thawing), followed by sonication for 2 min and centrifugation at 10,000 rpm for 30 min at 4 °C. Aliquots of the supernatant were taken to measure soluble protein. Fat was determined by Randall's method after 3 cycles of 15-s sonication. Moisture was determined by drying the sample in an oven at 105 °C until constant weight (AOAC 952.08, 2005). Total ash content was determined by heating the samples at 550 °C for 5 h in a muffle furnace (AOAC 838.08, 2005). Total fiber was determined by the enzymatic–gravimetric method. The total caloric strength was determined by multiplying the total protein, lipids and carbohydrate content values by 17, 38 and 17 kJ, respectively, and then summing the obtained results.

Protein characterization

The protein pattern of the biomass treated by freeze–thaw and sonication was evaluated with SDS–PAGE according to the method of Laemmli (1970), using 4% (w/v) stacking and 12% (w/v) separating gels. Samples were prepared, mixing them with sample buffer 4 × at 4:1 ratio, incubated in boiling water for 5 min and then centrifuged for 30 s at 8,000 rpm. The samples were subjected to a constant current of 120 V. After electrophoresis, the gel was stained overnight with 0.2% Coomassie Brilliant Blue R250 and distained with an ethanol: acetic acid solution. Molecular weight markers were used.

Fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) spectra were recorded with a Nicolet 6700 Thermo Scientific spectrometer at room temperature. Mid-infrared (MIR) spectra in ATR (attenuated total reflectance) mode were acquired over the range of 400—4000 cm^{-1} from 64 co-added scans at 4 cm^{-1} resolution. Powder samples were placed on a quartz crystal and pressed with a steel tip to ensure good contact.

Table 1 Formulation of breads

Ingredient g%	Control	AP1	AP5	AP10
Whole wheat flour	50	49	45	40
Arthrospira platensis	-	1	5	10
Yeast	0.4	0.4	0.4	0.4
Salt	0.6	0.6	0.6	0.6
Water	49	49	49	49
Total	100	100	100	100

AP1 Bread enrichment at 1% in *Arthrospira platensis*

AP5 Bread enrichment at 5% in *Arthrospira platensis*

AP10 Bread enrichment at 10% in *Arthrospira platensis*

UV–Visible spectrophotometry

UV–Visible spectra of algae extract samples both in pure aqueous and phosphate buffer medium were recorded with a diode array spectrophotometer (Agilent 8453) in the range 200–500 nm at room temperature. Solutions were diluted either with pure water or with phosphate buffer up to a suitable concentration and were placed in quartz cuvettes with 1 cm optical path for data acquisition.

Bread formulations

The experimental formulation of the developed samples of bread contained the typically used ingredients such as whole wheat flour, salt, dehydrated yeast and water. Dried microalgae biomass was added directly to develop a high protein bakery product as described in Table 1. Dry ingredients were mixed and then water was added, homogenized and kneaded. Kneading was performed using a Peabody brand stand mixer (PE-BM101 220 v) until the desired texture was achieved. The dough was then held for 30 min to leaven. The loaves were then baked at 220 °C for 35 min. Finally, the breads were removed from the hot molds and allowed to cool to room temperature.

To determine the nutritional composition of the samples of bread, moisture, ash, protein, carbohydrate and fiber contents were measured (AOAC 2005). To evaluate the effect of baking on proteins, in vitro protein digestion was determined and measured in baked bread samples according to De Marco et al. (2014) with slight modifications. Samples (3 g) were mixed with 80 mL of 0.2 mol L^{-1} HCl (pH 2.0) containing 0.05 mg mL^{-1} porcine gastric mucosal pepsin (Sigma-Aldrich) and incubated at 37 °C for 30 min. Then, 23 mL boric acid 1 mol L^{-1} and 0.5 mol L^{-1} NaOH were added, adjusted to pH 6.8 with 5 mol L^{-1} HCl and containing 0.25 mg mL^{-1} pancreatin.

The reaction took place at 37 °C in a stirring water bath over 120 min and was stopped with the addition of 1 volume of 20 g L^{-1} trichloroacetic acid (TCA). The samples were stored for 1 h, then centrifuged (8000 x*g*, 10 min, 25 °C) and the sediments were analyzed for nitrogen content, reporting the protein content of the digestion.

Protein digestibility (PD) is defined as the fraction (or percentage) of protein that is digested and is expressed as:

$$PD = \frac{(Total\ protein\ content - Protein\ content\ after\ digestion) \times 100}{Total\ Protein\ content}$$

Protein availability (PA) reflects the amount of protein in the bread that is available to be digested. The calculation of protein availability is as follows:

$$PA = \frac{PD \times Total\ protein\ content}{100}$$

Statistical analysis

The analyses were conducted in triplicate and the values are expressed as mean value ± standard deviation (Mean ± SD). Analysis of variance (ANOVA) was performed using the INFOSTAT software statistical 2017 (https://www.infostat.com.ar/). The multiple comparisons of means of each analysis used the Duncan test at the confidence level of 95%.

Results

Biomass productivity

Arthrospira platensis was grown for 75 days and the growth rate expressed as dry weight (g L^{-1}) was 0.58 g L^{-1} under a 24 h light photoperiod. This result is consistent with that reported by Aouir et al. (2017) for fresh starter cultures of *A. platensis* at 30 ± 2.0 °C, exposed under cool white fluorescent tubes with light/dark cycles of 12:12 with 35 µmol photons m^{-2} s^{-1} light intensity. On the other hand, Kumar et al. (2011) found similar growth for *A. platensis* cultures at 30 °C with a 12-h light/dark photoperiod provided by white fluorescent lamps.

Table 2 Chemical composition and energy content in *Arthrospira platensis* from Yaragui Lagoon

Sample	Protein[%]	Lipids[%]	Carbohydrates[%]	Moisture[%]	Ash[%]	Fiber[%]	Energy [kJ/100 g]
Arthrospira platensis	67.40 ± 0.31	4.71 ± 0.33	2.30 ± 0.43	14.76 ± 0.39	9.27 ± 0.04	1.56 ± 0.06	1342.7 ± 6.89

Data are presented as mean ± standard deviation (n = 2)

Table 3 Protein N, nitrogen-to-protein factor (NTP), total protein and water-soluble protein contents and proportion of water-soluble protein in content in *Arthrospira platensis* from Yaragui Lagoon

Sample	Parameter				
	Protein N Kjeldhal	NTP	Total protein [%]	Hydrosoluble protein [%]	Hydrosoluble protein/Total protein [%]
Arthrospira platensis	10.78 ± 0.05	6.25	67.40 ± 0.31	44.56 ± 2.54	66.11 ± 0.30

Data are presented as mean ± standard deviation (n = 2)

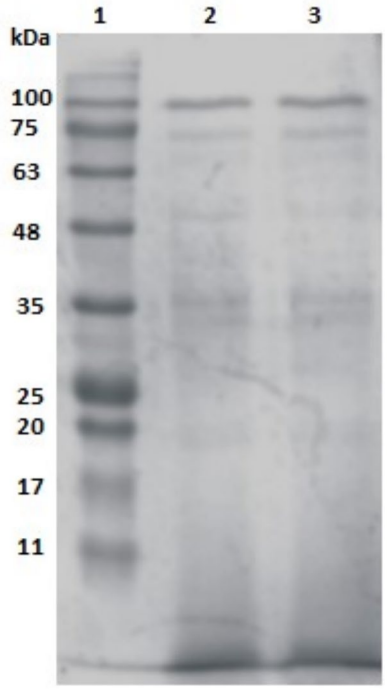

Fig. 2 SDS Page of *Arthrospira platensis* from Yaragui Lagoon

Proximate composition

Table 2 summarizes the proximate composition of the strain. As Table 3 shows, using a nitrogen-to-protein (NTP) conversion factor of N × 6.25, the protein content obtained was 67.40%.

Protein pattern

The protein profile of the treated biomass was determined by SDS-PAGE to analyze the molecular weight of all microalgal, intracellular and cell wall proteins. As shown in Fig. 2, a band around 19 kDa was observed, corresponding to α/β subunits of biliproteins. Two other bands at about 35 kDa were observed, probably corresponding to impurity proteins. The chlorophyll protein a little above of 47 kDa and a nuclear membrane linker protein (95 kDa) belonging to *A. platensis* were also observed. In addition, a band around 75 kDa was observed.

Fourier transformed infrared spectroscopy

FTIR spectrum of the sample obtained by ATR is shown in Fig. 3. Protein molecules show many vibrational features that can be assigned to several characteristic group frequencies arising from the polypeptide repeat unit, and they have been identified in this spectrum: 3075 cm^{-1} and 3269 cm^{-1}

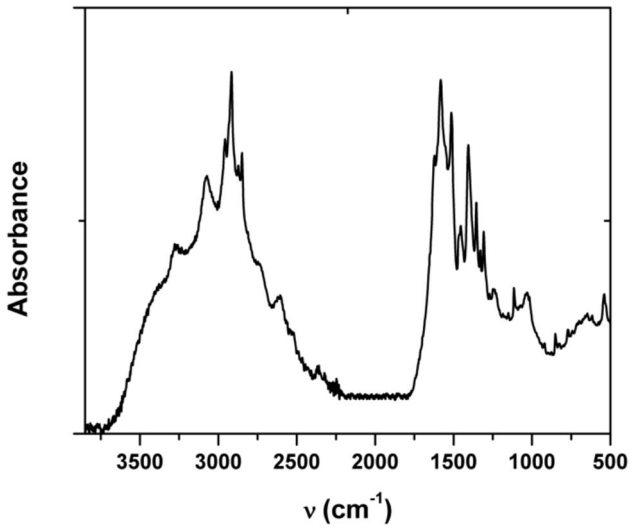

Fig. 3 FTIR spectra of *Arthrospira platensis* from Yaragui Lagoon

bands correspond to stretching vibrations of O–H bonds and stretching of N–H bonds (Amide A and Amide B bands), respectively. Peak at 2957 cm^{-1} is assigned to asymmetric stretching vibrations of C-H of CH_3 groups, while the one at 2916 cm^{-1} corresponds to asymmetric stretching vibrations of C-H of CH_2 groups. Also, 2873 cm^{-1} is ascribed to symmetric stretching vibrations of C-H bonds of CH_3 groups and 2848 cm^{-1} asymmetric stretching vibrations of C-H of groups CH_2O.

Amide I band is one of the most intense and characteristic absorption bands in proteins 1623 cm^{-1} (stretching of C = O bond), while another important band (Amide II) is related to stretching of C-N bonds (1583 cm^{-1}) and bending vibration of N–H bonds (1455 cm^{-1}).

UV–Visible spectrophotometry

UV–Visible spectra for aqueous (a) and phosphate buffer (b) samples are shown in Fig. 4. In both spectra, there is an intense and sharp peak at 210 nm, and also a wide and less intense band at 280 nm.

Bread nutritional properties and protein digestibility

Table 4 shows that the nutritional composition of the pieces of bread analyzed had no significant differences in ash and moisture content. In all bread samples the main ingredient was wheat flour, thus in all formulations lipid contents were low and carbohydrate contents were high, according to the composition of the principal raw material.

No significant differences were found between the control bread and the breads fortified with 1% and 5% of *S. platensis*. The in vitro protein digestibility percentage of samples of bread enriched with 1% *A. platensis* did not show significant differences with the control bread, in accordance with the protein content of both, while bread formulated with 5% enrichment showed higher digestibility values than the control bread and the bread enriched with 1% *A. platensis*.

Discussion

Biomass productivity

Several authors have reported that the nutrient status is the main factor which has an impact on the growth and productivity of microalgae cultures (Jourdan 2011; Kumar et al.

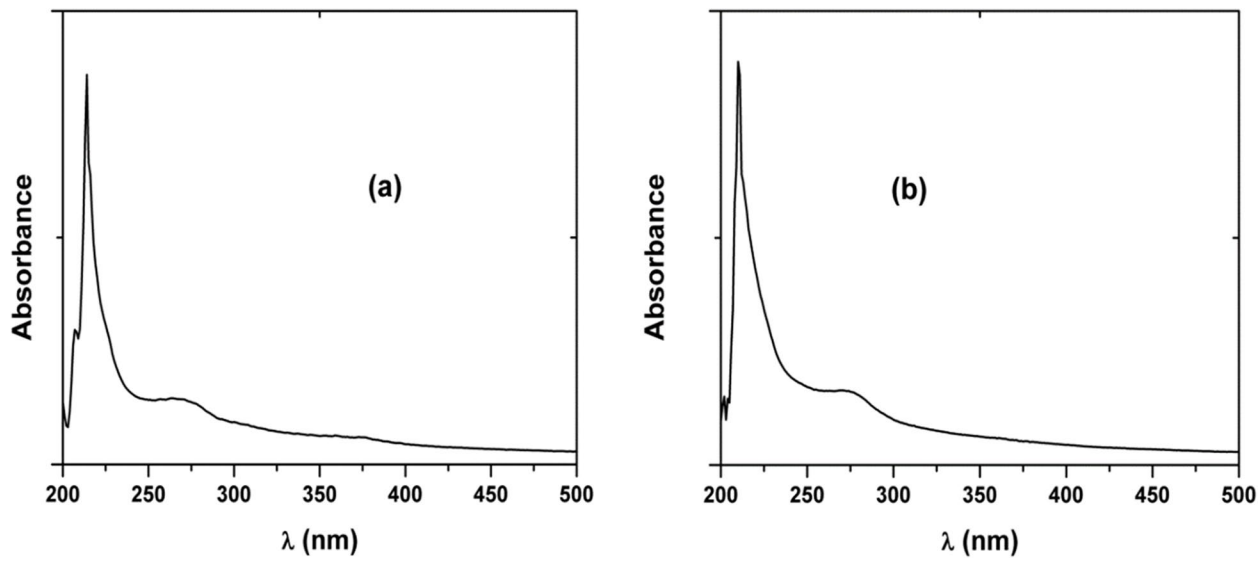

Fig. 4 UV–Visible spectra for aqueous (**a**) and phosphate buffer (**b**) samples of *Arthrospira platensis* from the Yaragui Lagoon

Table 4 Chemical parameters of the bread formulates

Sample	Protein [%]	Lipids [%]	Carbohydrates [%]	Moisture [%]	Ash [%]	Fiber [%]	Energy [kJ/100 g]
Control	7.04 ± 0.77[a]	0.96 ± 0.07[a]	41.80 ± 0.38[a]	46.33 ± 0.77[a]	1.91 ± 0.07[a]	1.96 ± 0.04[a]	852.47 ± 5.77[a]
AP1	6.94 ± 0.40[a]	0.89 ± 0.03[a]	43.34 ± 1.46[a]	44.29 ± 2.18[a]	2.10 ± 0.60[a]	2.43 ± 0.17[ab]	874.29 ± 29.91[a]
AP5	7.02 ± 0.28[a]	0.86 ± 0.15[a]	41.95 ± 1.54[a]	45.74 ± 0.81[a]	2.10 ± 0.46[a]	2.31 ± 0.13[ab]	851.51 ± 26.63[a]
AP10	8.62 ± 0.71[b]	0.73 ± 0.20[a]	41.62 ± 3.71[a]	43.74 ± 2.20[a]	2.68 ± 0.26[a]	2.60 ± 0.36[b]	867.52 ± 43.05[a]

Data are presented as mean ± standard deviation (n = 2). Data within a column followed by different small letters are significantly different by the Duncan test ($p < 0.05$).

[a] it means the minor value,

[b] it means the major value

2011; Madkour et al. 2012; Ould Bellahcen et al. 2013). Environmental factors affecting microalgal growth include temperature, pH, salinity, inorganic carbon availability and light, which is one of the key factors controlling the course of microalgal physiological processes. The quantity and quality of light determine the amount of energy available for photosynthesis. The light/dark regime that affects algal growth and biomass production is equally important (Krzemińska et al. 2014). In a natural environment, light intensity is constantly changing and the light regime is not constant (Khoeyi et al. 2012). Changes in the biochemical composition of microalgae are induced by changes in the amount of light. Increasing the frequency of light/dark cycles can significantly improve productivity and photosynthetic efficiency (Grobbelaar 2009). Therefore, it is necessary to provide light of an appropriate intensity, duration and wavelength (Carvalho et al. 2011; Pedrosa-Bezerra et al. 2012). Specifically, light duration is the key factor in microalgae growth because the way in which microalgae behave in terms of photosynthesis is affected by light conditions (Benavente-Valdés et al. 2016). It is known that biomass production can be negatively affected by the dark phase of the culture process due to respiratory loss, with up to 35% of the biomass accumulated during the light phase being lost (Sui et al. 2019). The rate and extent of biomass loss during the dark phase depend on the microalgae species and the specific culture conditions (Edmundson and Huesemann 2015). In addition to biomass loss, the light/dark cycle also induces changes in the macromolecular composition of the cell's biochemistry. For instance, energy storage compounds such as carbohydrates accumulate during the light phase and decrease during the dark phase for use in nocturnal metabolism, e.g. protein synthesis. This affects the levels of these macronutrients (de Winter et al. 2017; Hidasi and Belay 2018). Furthermore, smaller molecules that comprise macromolecules, such as amino acids and fatty acids, are also affected (Sui et al. 2019).

This study examined biomass production using a 24-h photoperiod because a previous screening found that microalgal growth benefited from a longer illumination period when using a 12:12 light/dark cycle. This resulted in a higher specific growth rate and biomass concentration with a 24-h light cycle than with the 12:12 light/dark cycle (data not published). Several studies have evaluated the effect of photoperiod on biomass production, providing valuable insights into this process. For instance, Jacob-Lopes et al. (2009) examined the impact of photoperiod on the growth of the cyanobacterium *Aphanothece microscopica* and observed a linear reduction in biomass production as the light period shortened. They also found that 12:12 cycles (light/dark) were an exception to this reduction, determining that a 24-h light photoperiod was the most effective. The effects of photoperiod on the growth rates of *Chlorella vulgaris* were also investigated, revealing that the maximum growth rate occurred at a 16:8-h light/dark photoperiod (Seyfabadi et al. 2011). This study also reported that total protein content differed significantly between light regimes, with the highest percentage (46%) occurring at a 16:8-h light/dark photoperiod and the lowest percentage (33%) occurring at an 8:16-h light/dark photoperiod. This is consistent with the data obtained in the present study, since Villalba et al. (2018) studied the same strain in the same location and obtained a lower protein content using a 12:12 light/dark photoperiod. In contrast, the present research obtained a protein content of over 65% using a 24-h light photoperiod.

Proximate composition

It is normally assumed that the differences in protein content among the microalgal species are primarily due to the differences in their genetic characteristics. However, the reasons for the different protein content of a given species are multifaceted, including the method used to analyze the protein, the environmental conditions under which they were grown, and the growth stage at which the microalgal biomass was harvested (Wang et al. 2021). Using an N to P conversion factor of 6.25 the protein content obtained was 67.40%. However, using the same method of quantification, Villalba et al.

(2018) found a lower percentage of protein for the same strain. This discrepancy could be due to the different environmental conditions in which they were grown, since the photoperiod and the culture medium were different. Also, Villalba et al. (2018) applied the conversion factor of N × 5.95. The non-protein nitrogen content of microalgae ranges from 4 to 40%, depending on the species, season and growth stage (Lourenço et al. 2004; Tibbetts et al. 2015). In general, this content is due to many nitrogen-containing components, such as chlorophyll, nucleic acids amines, glucosamides and pigments, which are commonly expected to be around 10% of the total nitrogen in microalgae (López et al. 2010; Templeton and Laurens 2015). The protein content of *Arthrospira* strains studied by Aouir et al. (2017) was 60% for the strain grown in pond culture. Bensehaila et al. (2015) reported a protein content of 60.32%. Both studies reported lower values than the 67% observed in the present work. On the other hand, the value obtained is in accordance with the protein content reported by Tokuşoglu and Ünal (2003). Nevertheless, the protein content obtained is higher than that of many other dietary protein sources. It has also been previously reported that the protein content of *A. platensis* has a very high digestibility (75–83%) due to the lack of cellulose cell walls, making it a preferred dietary source for obtaining high protein (Aouir et al. 2017).

Despite the high protein content of microalgae, the presence of a cell wall requires a modification step for their extraction and that of other macronutrients and biocompounds. After cell rupture, soluble protein measurements were performed and an overall experimental Kjeldahl nitrogen to protein conversion factor of 6.2508 was determined with a correlation coefficient of 0.9677.

Total lipid content obtained was lower than those reported by Tokuşoglu and Ünal (2003) and Bensehaila et al. (2015). The fresh cells of *Arthrospira* reported by Aouir et al. (2017) showed a similar value of lipids to that obtained in this work, while the other ones were higher. This difference is closely related to the differences in pH, temperature and light in the culture conditions (Kumar et al. 2011). Richmond (2004) reported that the lipid production of microalgae depends on the species and its culture conditions such as nutrients, salinity, light intensity, temperature, pH and even the association with other microorganisms. In addition, lipid accumulation is increased when there is a nutrient deficiency in terms of nitrogen and phosphorus (Bhakar and Pabb 2014). The total carbohydrate content was 2.3%. Higher contents, averaging 15%, have been reported by other authors (Tokuşoglu and Ünal 2003; Bensehaila et al. 2015; Aouir et al. 2017).

The moisture content of the samples was 14.76 ± 0.39%. This is slightly high as the recommended condition for long term storage of powders indicates that it should be less than 10% (Aouir et al. 2017). The ash content was consistent with that reported by Chagas et al. (2016) (7.94%) and Gai et al. (2013) (9.6%) for *A. platensis*. On the other hand, it is slightly higher than the ash content reported by Almeida (2017) for *A. platensis* (6.37%). The content of dietary fiber in seaweed is an important nutritional parameter that promotes beneficial physiological effects, such as laxation and the attenuation of cholesterol and glucose levels in blood (Mišurcová et al. 2010). They contain compounds such as polysaccharides, oligosaccharides, lignin and associated phytochemicals, which are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine (Praznik et al. 2014). The fiber content obtained was 1.56%. This result is lower than the results of Koru (2009) and Mišurcová et al. (2010), who found fiber content of about 3% in the same culture species. Finally, total energy content value was low due to the small amounts of carbohydrate and lipid in its composition.

Protein pattern

Proteins are present in the cell wall, cytoplasm, chloroplast and moreover in all organelles of algae (Safi et al. 2015). The protein profile of the treated biomass was determined by SDS-PAGE to analyze the molecular weight of all microalgal, intracellular and cell wall proteins. As shown in Fig. 2, a band around 19 kDa was observed, corresponding to α/β subunits of biliproteins (Kamble et al. 2013; Rajakumar and Muthukumar 2018). Two other bands at about 35 kDa were observed, probably corresponding to impurity proteins. The chlorophyll protein a little above of 47 kDa and a nuclear membrane linker protein (95 kDa) belonging to *A. platensis* were also observed (Sudhir et al. 2005; Rajakumar and Muthukumar 2018). In addition, the presence of a band around 75 kDa is consistent with the crude extract analyzed by Chia et al. (2020) of sonicated *Arthrospira* in phosphate buffer.

Fourier transformed infrared spectroscopy

Characterization of proteins from *A. platensis* by FTIR was successful, since every peak present in the spectrum could be assigned to a characteristic functional group of a protein. FTIR spectrum also provides insight into the secondary structure of proteins. Amide I band (1600–1720 cm^{-1}) is related to C = O stretching while Amide II band (1480–1575 cm^{-1}) is associated with N–H bending (Kong and Yu 2007; Theivandran et al. 2015). Since both C = O and N–H are involved in hydrogen bonding among different parts of the protein molecule the positions of both Amide I and Amide II bands are sensitive to the secondary structure composition of a protein (Yang et al. 2015). Amide I band in a α-Helix structure shows a frequency range of

1648–1657 cm^{-1}, while in β-sheet structure corresponds to a range of 1623–1642 cm^{-1} (Adochitei and Drochioiu 2011; Cobb et al. 2020). Since in our spectrum 1623 cm^{-1} peak is considerably more intense than the 1651 cm^{-1} peak, which is only a small shoulder, we may infer the main secondary structure of *A. platensis* is β-sheet structure.

UV–Visible spectrophotometry

UV–Visible spectra for aqueous (a) and phosphate buffer (b) samples are shown in Fig. 4. In both spectra, there is an intense and sharp peak at 210 nm, which is present in all protein spectra and it is ascribed to the absorption of the peptide bond (Becklin and Desiderio 1995; Pereverzev et al. 2018). Proteins also absorb light at 280 nm, with peak height dependent upon the fraction of the aromatic amino acids, tryptophan and tyrosine (and to a small extent on the amount of phenylalanine and disulfide bonds), within the protein (Biter et al. 2019). Since this band is present in both spectra with moderate intensity, we may infer that protein extracted from *A. platensis* has a sizeable fraction of these amino acids in its composition.

Bread nutritional properties and protein digestibility

Bread is a universally consumed food, which makes it a suitable product to be enriched in protein. The partial replacement of wheat flour with *Arthrospira* in the bread formulations resulted in significant improvements in the protein content of the product when the total content was 10%. The improvement in protein content is due to the high concentration of these macromolecules in the microalgae biomass. Unlike most algae, *Arthrospira* has a high digestibility due to the lack of cellulose in its cell wall (De Marco et al. 2014). This facilitates its use for human consumption (Tomaselli 2004). On the other hand, in vitro digestibility provides useful information on product nutrient bioavailability, in this case proteins. The in vitro protein digestibility of samples of bread enriched with 1% *A. platensis* did not show significant differences with the control bread, by the protein content of both. The bread formulated with 5% enrichment showed higher digestibility values than the control bread and the bread enriched with 1%. These data suggest that although the protein content was not affected in quantity at this level of fortification, the quality of the protein provided was enough to show a statistical difference in digestibility. With the 10% *A. platensis* formulation, digestibility increased dramatically to over 90%, which is more than double that of the control bread. This would indicate that the *A. platensis* protein has a digestibility significantly higher than that of the main raw material of the developed product. These results are consistent with those reported by Niccolai et al. (2019) for *A. platensis* protein (approximately 80%). Mišurcová et al. (2010) also reported in vitro digestibility values for *A. platensis* biomass of approximately 85% using pepsin and pancreatin. In contrast, De Marco et al. (2014) observed a decrease in digestibility of *A. platensis* enriched pastes using similar concentrations and the same digestion protocol. As can be seen in Fig. 5, the most notable feature of the 10% enrichment was that it combined high digestibility with high availability, so that almost all of the protein was available.

Conclusions

The studied *Arthrospira platensis* from Yaragui Lagoon of the Paraguayan Chaco showed a high content of total proteins, of which approximately 66% corresponds to the hydrosoluble fraction. The analysis of protein nitrogen allowed the experimental determination of the nitrogen-to-protein conversion factor. The protein analyses of the formulated

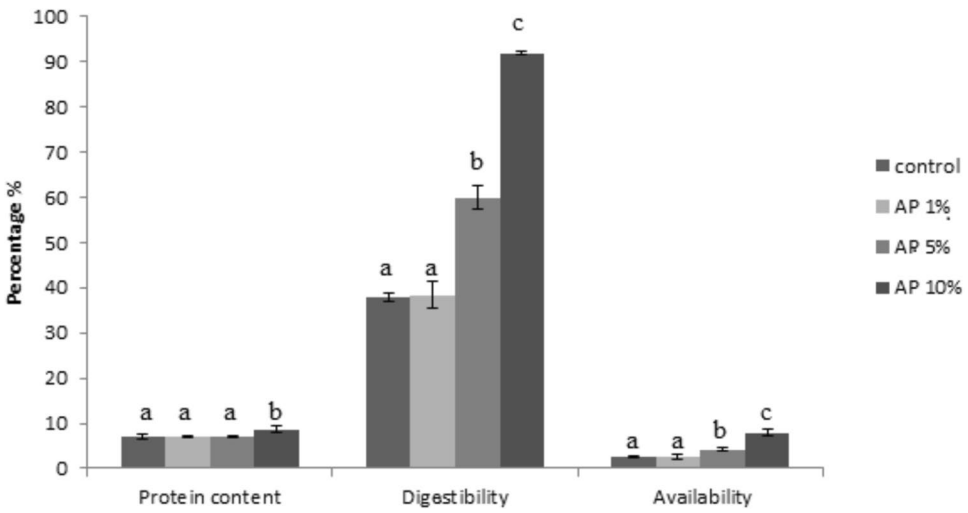

Fig. 5 Digestibility and availability of bread protein content enriched with *Arthrospira platensis* from the Yaragui Lagoon of the Paraguayan Chaco. Results are means ± standard deviation (n = 2). The data for each parameter indicated by different small letters are significantly different by Duncan's test ($p < 0.05$)

breads indicate that a fortification of more than 5% is necessary to obtain protein values significantly higher than those present in the original food. However, the digestibility of the proteins of this strain is considerable and with a fortification higher than 5% it was possible to double the digestibility. In addition, the use of microalgae protein represents an emerging technological alternative that has no negative impact on the environment. Therefore, the Yaragui Lagoon could provide an economic benefit. In this sense, this work provides new information and the results suggest that the use of the strain studied is a viable alternative of triple impact. Finally, it is important to mention that this study is in line with several Sustainable Development Goals (SDGs) proposed by the United Nations.

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Author's contribution RP: Methodology, formal analysis, data curation, conceptualization. MV: Methodology, formal analysis, validation. VAE: methodology, formal analysis, visualization, validation. IDE: Methodology, formal analysis Review & Editing. NC: Methodology, formal analysis. DL: Methodology, formal analysis, writing—original draft, visualization, validation, investigation, review & editing, supervision, methodology, conceptualization.

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Data availability Data will be available on request.

Declarations

Competing interests The authors declare no competing interests.

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