



GROWTH, NUTRITIONAL QUALITY AND PIGMENTS CONTENT OF *ARTHROSPIRA PLATENSIS* CULTIVATED IN MEDIA BASED ON *PENNISETUM PURPUREUM* (SCHUMACH) STEMS CARBONATED EXTRACTS

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Abstract: The present study aimed to evaluate *Penisetum purpureum* stems carbonated extracts' contribution to the growth, nutritional quality and general pigment content of *Arthrospira platensis*. To this end, 6 experimental culture media were developed, including 5 based on *Penisetum purpureum* stems carbonated extract at the rate of 300g/l, 350g/l, 400g/l, 450g/l and 500g/l and 1 reference medium. Pure *Arthrospira platensis* strain was inoculated at 5ml/l in each medium and incubated. The physicochemical parameters of media were monitored daily and biomass was harvested weekly to assess production. Mineral characterisation of extract and the post-harvest biomass, nutritional and pigment content of each. The results show that *Pennisetum purpureum* stems carbonated extract contains several minerals, particularly K, S at the rate of 58474.32 and 4786.099 ppm respectively, and Rh, Fe, Cu, Br and Rb. *Pennisetum purpureum* stems carbonated extract did not significantly influence biomass production however, the 500g/l rate of *Pennisetum purpureum* stems carbonated (M6) revealed a substantially higher effect on biomass production, carbohydrate and lipid content compare to the others. The best record was registered concerning protein and iron content at the rate of 450g/l of *Pennisetum purpureum* stems carbonated (M5). The general pigments analysis of the post-harvest biomass from the different experimental media revealed a significantly higher effect of the culture medium at a dose of 500g/l (M6) of *Pennisetum purpureum* stems carbonated extract compared with the other culture media. Chlorophylls A and B, carotenoids and phycocyanine content was recorded at the rate of $4.9 \pm 1.8\text{mg/g}$, $5.65 \pm 1.05\text{mg/g}$, $10.82 \pm 0.0 \text{ mg/g}$ and $0.95 \pm 0.01 \text{ mg/g}$ respectively.

Keywords: *Arthrospira platensis*, *Penisetum purpureum*, nutritional quality; general pigments

I. INTRODUCTION

The cyanobacterium *Arthrospira platensis*, also known as *Spirulina platensis*, is a photoautotrophic filamentous microalga consumed for centuries by certain primitive peoples in Africa and America. It has been attracting growing interest from the international scientific community for several decades due to its nutritional value and potential use as a source of therapeutic and curative properties (Sguera, 2008). *Arthrospira platensis* is characterised by the production of a wide variety of interesting substances, mainly proteins of very high nutritional value. It is therefore a non-conventional food source of high nutritional quality, thanks to its high digestibility, high protein content (10 to 11% of its wet mass and 50 to 70% of its

dry weight), richness in essential fatty acids, vitamins and major minerals (Sguera, 2008). Its potential seems even greater in view of its richness in essential pigments, the main one being phycocyanin, which gives spirulina its characteristic blue-green color. Spirulina's concentration of these photosynthetic pigments exceeds 20% of dry weight, and includes phycocyanin, chlorophylls, beta-carotene and xanthophylls, all of which can have beneficial and commercial value (Vonshak, 2002 and Koru, 2009). Chlorophyll is the most visible pigment in spirulina, contributing between 6.8 and 11 g kg⁻¹. It releases ions when struck by the energy of sunlight. These free ions stimulate the biochemical reactions that form the proteins, vitamins and sugars in spirulina cultures (Rangel-Yagui et al., 2004). Carotenoids, for their part, are generally responsible for the red and yellow hues observed in nature, and average between 3.4 and 4.0 g kg⁻¹. Beta-carotene accounts for 80% of the carotenoids present in spirulina, which is convertible into vitamin A (Vonshak et al., 1996; Habib et al., 2008; Theodore and Georgios, 2013). Xanthophyll is present in spirulina at a concentration of 1.0 g kg⁻¹ and its concentration depends on the species and environmental conditions. Xanthophylls are an important source of yellow-orange pigments. They are used in the poultry industry as a food additive to improve the highly coloured egg yolk, as well as the pigmentation of meat and skin that appeal to consumers (Durand-chastel, 1980; Richmond, 1988). Phycocyanins, on the other hand, are an important source of blue pigment for use in food colouring, and are found in spirulina at a concentration of between 30 and 220 g kg⁻¹ (Fairchild and Glazer, 1994). In addition to its nutritional properties, some studies have also highlighted its activities on the immune system, cancer and AIDS, as well as its effects on the fight against cell ageing, and its hepatoprotective and anti-inflammatory properties (Sguera, 2008).

Spirulina is being developed for cultivation in the regions where it occurs naturally, in Africa, Asia and America, as well as in farms specially designed for its production on an industrial scale. Its low-cost production is necessary when considering large-scale cultivation for industrial purposes. The cost of nutrients is considered to be the second major factor influencing the cost of producing Spirulina biomass after labour (Vonshak, 1997). Zarrouk's medium was successfully used as a standard medium (SM) for its culture for many years (Zarrouk, 1966). However, the production of these cyanobacteria in this synthetic medium is very costly due to the high mineral requirements of this alga, the rapid depletion of minerals and the difficulty in maintaining favorable culture conditions for *Arthrospira platensis* (Benahmed & Benamara, 2013). The unavailability and high cost of certain chemical inputs on the local market is an obstacle to the mass cultivation of this seaweed, hence the need to use other mineral sources. It is therefore important to look for other culture substrates that will enable these microalgae to be mass-produced at low cost, as well as the mineral elements that it will use up in this culture medium if the culture techniques and conditions are properly controlled. In recent years, research has focused on so-called non-conventional resources which may provide opportunities for the development of efficient culture media for the production of *Spirulina platensis*. These non-conventional biological mineral sources, which are alternatives to synthetic mineral sources that could potentially be used for Spirulina cultivation, include agricultural by-products and edible plants, because of their mineral potential. To this end, *Pennisetum purpureum* (Poaceae) stems carbonated extracts are interesting opportunities to explore.

Also known as elephant grass, *Pennisetum purpureum* is a large perennial plant in the grass family, 2 to 4.5 meters high, exceptionally 7.5 meters, with leaves 30 to 120 cm long and 1.5 centimeters wide. Elephant grass is native to tropical sub-Saharan Africa (Clayton et al., 2013; cited by Heuzé, 2020). It has been introduced as fodder in most tropical and subtropical regions of the world. In Cameroon, this plant is used not only as a fodder grass, but also as an organic fertilizer, thanks to its high potassium content (Tchoumi et al., 2017). Previous studies carried out by Lin (2006) on the mineral characterization of some strains of *Pennisetum purpureum* have highlighted the presence of major mineral elements such as magnesium, phosphate, potassium, calcium, iron, sodium and many others. However, the use of extracts from *Pennisetum purpureum* strains as mineral sources in Spirulina cultivation has not yet been scientifically investigated. Hence the interest of this scientific study, which aims to assess the effect of culture media, based on *Pennisetum purpureum* stems carbonated extract on the growth, nutritional quality and pigment content of *Arthrospira platensis*.

II. RESEARCH METHODOLOGY

II.1. Experimental site

The study took place in the locality of Yabassi, the capital of the Nkam Department, one of the four departments of the Littoral Region of Cameroon. It is located between latitudes 9°50' and 10°10' North, and between longitudes 4°20' and 4°40' East, with an average altitude of 15 to 20 m, corresponding to the NKAM valley. The climate in the Yabassi area is sub-equatorial with tropical tendencies, with two seasons: a dry season from November to June and a rainy season from July to October. The temperature ranges from 24.9°C to 28.2°C, with an average of 27.5°C.

II.2. Microorganism

The *Arthrospira platensis* cyanobacteria used in this experiment is the Toliarensis strain from the salt lakes in the Tuléar region of Madagascar. It was purified and maintained in culture at the *Spirusud-antenna* production farm based in Toliara. Once at the Spirulina Pilot Production Unit of the Institute of Aquatic Sciences of the University of Douala at Yabassi, the inoculum was cultured in a new culture medium (modified Jourdan (1996) synthetic medium) with the following composition (g/l): Sodium bicarbonate (NaHCO_3), 08; Trisodium phosphate (P_2O_5), 0.2; Potassium sulphate (K_2SO_4), 0.1; Magnesium sulphate (MgSO_4), 0.1; Potassium nitrate (KNO_3), 0.2; Iron sulphate (FeSO_4), 0.02; Sodium chloride (NaCl), 05; Calcium chloride (CaCl_2), 0.02; Natron, 05; Green clay, 02. The culture was carried out by variable geometry as recommended by Jourdan (1996) until 50 l of pure culture was obtained.



Figure 1 : *Arthrospira platensis* strain

II.3. Preparation of *Pennisetum purpureum* stems carbonated extract

Pennisetum purpureum stems carbonated extract was prepared using the method described by Jourdan (2014). To this end, 5 kg of *Pennisetum purpureum* stems were harvested in situ near Campus II of the Institute of Aquatic Sciences of the University of Douala at Yabassi. Once harvested, they were washed and drained. At the end of this phase, the stems were dried in the shade for a fortnight. Once dried, the stems were weighed and then calcined until 2.1kg of ash was obtained. The ash obtained was then leached with 15 l of water through a suitable mechanical filtration device. The lye thus produced was collected after 24 hours, and then left to carbonate for 15 days before use, by stirring the substrate at regular intervals to encourage the renewal of the air in the substrate.

II.4. Identification and quantification of mineral constituents of *Pennisetum purpureum* stems carbonated extract

The mineral constituents of *Pennisetum purpureum* stems carbonated extract were identified and characterized using energy dispersive X-ray fluorescence spectrometer (EDX-7000, Na-U, Shimadzu, Japan) with liquid method, calibration with Al-Cu standard (Chai *et al.*, 2017; Nyakuma *et al.*, 2021). 5–10 mL of extract sample in replication of three was placed over a thin film lined a 10 mL Polypropylene cup and then mounted inside the EDX-7000 spectrometer (Khan *et al.*, 2021; Yousaf *et al.*, 2017). The instrument is equipped with an X-ray tube using a Rhodium (Rh) target and a high-performance silicon drift detector (SDD), operated with a maximum of 50 kV and 1000 μA and a PCEDX-Navi software. The elemental composition of the sample was detected under an air-based atmosphere. The analytes were then assessed with a collimator of 10 mm in diameter with a live acquisition time of the 60 s (Bilo *et al.*, 2015). During the analysis, the sample was irradiated with X-rays. The incident beam ejected an electron from inside the atom, which was replaced by an electron from the top layer. This transition emitted a photon whose energy corresponded exactly to the gap between the two electron levels. By measuring the energy of this photon, the emitting atom was identified (Rohit Kumar *et al.*, 2021). Once the measurement was complete, the names of the elements and their various proportions were displayed. The results obtained using the XRF method are presented in values of irradiation intensity expressed in cps/ μA (cps is the line intensity in standard units (counting per second), μA is the current intensity).

II.5. Culture media

06 experimental culture media were prepared for this study using the method described by Jourdan (1999). The different proportions of inputs used to make up the different experimental media are summarized in Table I.

Table 1: Composition of experimental culture media.

Inputs	Culture media (g/l)					
	M ₁	M ₂	M ₃	M ₄	M ₅	M ₆
Sodium bicarbonate (NaHCO ₃)	08	-	-	-	-	-
Trisodium phosphate (P ₂ O ₅)	0,2	-	-	-	-	-
Potassium sulphate (K ₂ SO ₄)	0,1	-	-	-	-	-
Magnesium sulphate (MgSO ₄)	0,1	-	-	-	-	-
Potassium nitrate (KNO ₃)	0,2	-	-	-	-	-
Iron sulphate (FeSO ₄)	0,02	-	-	-	-	-
Sodium chloride (NaCl)	05	05	05	05	05	05
Calcium chloride (CaCl ₂)	0,02	-	-	-	-	-
Natron	05	05	05	05	05	05
Green clay	02	02	02	02	02	02
<i>Pennisetum purpureum</i> stems carbonated extract	-	300	350	400	450	500

M₁ = Modified Jourdan medium; M₂ = Culture medium based on *Pennisetum purpureum* stems carbonated extract at a dose of 300g/l; M₃ = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at a dose of 350g/l; M₄ = Culture medium based on *Pennisetum purpureum* stems carbonated extract at 400g/l; M₅ = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at 450g/l; M₆ = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at 500g/l.

II.6. Experimental design

The experimental design consisted of a two-slope traditional greenhouse with a one-meter-high shelf inside. Eighteen (18) basins, each with a capacity of 20 liters, were used for the trials. They were arranged randomly on a shelf designed and assembled using Chinese bamboo. The various experimental media and the synthetic control medium were inoculated with a *Spirulina* pellet harvested by filtration from a young culture from a 7 m² production tank. 18 20-litre tanks were used for these experiments. Each experimental tank contained a previously prepared culture medium and was inoculated with 5ml of inoculum. Everything was mixed gently to homogenize the different media. Physico-chemical parameters such as temperature, pH, dissolved oxygen, conductivity, transparency and depth were measured daily using a multi-parameter meter, a Secchi disc and a graduated ruler. The culture media were stirred manually, as recommended by Jourdan (1999), on a daily basis at the following intervals: 8 am, 12 noon and 4 pm. Shaking was used to homogenize and ensure a good distribution of nutrients and light in the culture media; this allowed rapid alternation of light and shade on the *Spirulina* filaments. The photoperiod was 12 h/24 h.

II.7. Growth evaluation: Dry weight

The biomass concentration of each culture medium was assessed at the end of the harvest, which was carried out every 7 days at 7 a.m. precisely throughout the duration of the experiment. 500 ml of each culture medium was taken and then filtered using Whatmann filter paper, which had been weighed beforehand. The whole batch was then rinsed with 25ml of acidified distilled water (pH 4) to release all the salts and nutrients. After filtration, the filter paper was dried in a solar dryer at room temperature (35°C on average) and then reweighed. The dry weight was evaluated in g.l-1 (AOAC, 2000).

II.8. Nutritional characterization and quantification of general pigments in post-harvest biomass

II.8.1. Nutritional characterization of post-harvest biomass

II.8.1.1. Total proteins contents

Protein quantification was carried out using the method described by Devani *et al.* (1989), based on the principle of determining the total nitrogen content of the mineralization previously obtained from the various dry *Spirulina* samples. Ammonia (NH₃) reacts with acetyl acetone and formaldehyde in an aqueous medium to form a complex, 3,5-diacetyl-1,4-dihydrolutidine, which is yellow in color and absorbs with a maximum at 412 nm within the limits of 0.5 to 6 µg of nitrogen/ml. The intensity of the coloration is proportional to the amount of nitrogen present in the medium. 1.2 ml of sodium acetate was added to a test tube containing 0.5 ml of the previously obtained mineralisate, followed by 1.6 ml of reagent solution. The whole mixture was homogenised and incubated in boiling water for 15 minutes before being cooled to 30°C

in a stream of cold water. After incubation, the density obtained was read at 412 nm using a spectrophotometer against a blank consisting solely of sodium acetate and reagent solution. Three replicates were performed for each sample. Calibration was carried out with a solution of (NH₄)₂SO₄ (0.4mg/l).

$$\text{Nitrogen content: } N(\%) = [(V - V_0) / M \times V_1] \times 0.14$$

$$\text{Protein content: } P(\%) = \%N \times 6.25.$$

II.8.1.2. Fat contents (NF V 03-905)

Fat determination was carried out using the Soxhlet extraction method as described in the AFNOR standard (1977), using hexane as the solvent. To this end, 50 g of sample was placed in a Soxhlet and 500 mL of hexane was introduced into the flask. With the temperature set at 60°C, most of the solvent was removed using the rotary evaporator to avoid boiling the oil, which could alter the acidity values over time. The flask containing the lipids was placed in an oven for 30 min at 103°C, then in a desiccator for 30 min. The weight of the lipids was obtained by the difference between the final weight and the initial weight of the flask. The results were given by the following formula: **Fat content (% DM) = (A-B) .100/C. DM/100**

A: weight of flask + extract in grams; B: weight of empty flask in grams; C: weight of test sample in grams; DM: dry matter in percentage.

II.8.1.3. Carbohydrates contents (Miron, 2003)

Carbohydrate quantification was carried out using the method described by Miron (2003) based on the principle of the sulphuric acid-anthrone reaction adapted to algal biomass. To 100 mg of biomass, 8 ml of perchloric acid was added and the mixture was shaken vigorously and left to stand. After this phase, 5 ml of the freshly prepared anthrone reagent was added to 1 ml of the filtrate previously obtained, and then heated at 100°C for 12 minutes. A green color developed due to the formation of a glucose-anthrone complex. The optical density was then determined at 630 nm after cooling the mixture. The blank consisted of 5 ml of reagent added to 1 ml of distilled water. A standard curve was made by preparing known concentrations of D+ glucose dissolved in distilled water. Optical density and glucose concentration (Cg; mg/ml) are related by the following relationship: **Cg = 0.536 x OD 630 + 0.0028; Cg: glucose concentration (mg/mL).**

II.8.1.4. Energy value

The energy value of Spirulina was calculated from the analytical values for proteins, fats and carbohydrates from a packaged quantity of Spirulina. Using the average physiological energy values, the theoretical energy value of Spirulina was determined according to the following equation: **Energy value in Kcal = 4 carbohydrates + 4 proteins + 9 fats**

II.8.1.5. Iron content

Iron levels were determined by flame atomic absorption spectrometry using the AOAC method (2012).

II.8.2. Quantification of general pigments in post-harvest biomass

The main pigments investigated in this experiment were chlorophylls (A and B), carotenoids and phycocyanin.

II.8.2.1. Quantification of Chlorophylls

The various chlorophyll contents of the samples were determined spectrophotometrically after absolute extraction in methanol using the absorption coefficient factor reported by Vonshak (1997) by measuring the optical density at 645, 652, 663 according to the following Kitney (1941) equation: Total chlorophyll = 20.2 OD 645 nm + 8.02 OD 663 nm (mg/l solution); Chlorophyll a = 12.7 OD 663 nm - 2.8 OD 645 nm (mg/ml solution); Chlorophyll b = 22.9 OD 645 - 4.68 OD 663 nm (mg/ml solution).

II.8.2.2. Quantification of carotenoids

The method used to estimate carotenoids was spectrophotometry after extraction in 90% acetone (Vonshak & Borowitzka, 1991). The carotenoid content was determined by adding 25 ml of acetone to a 3 g sample and keeping it for 24 h in the refrigerator. The supernatant was centrifuged and 0.5 ml of aliquot was diluted with acetone. Taking the DIL as the dilution factor, the optical density (OD) of the solution was measured at 450 nm and the carotenoid content expressed as follows: Carotenoid content (%) = [OD 450 x DIL x C] / 2.8.

II.8.2.3. Quantification of phycocyanin (%)

Phycocyanin was determined according to Bennett and Bogorad (1973). For phycocyanin determination, 3g of sample solution was centrifuged and decanted. From the supernatant, 0.5 ml of solution was diluted 100-fold with distilled water. The optical density (OD) of each sample was measured spectrophotometrically at 615 and 652 nm. The phycocyanin content expressed as % of dry matter (DW) was

estimated using the following relationship: Phycocyanin content (%) = $[1.873 \times (\text{OD } 615 - 0.473 \times \text{OD } 652) \times 100] / C$; C being the concentration of Spirulina in the solution.

II.9. Statistical analysis

Results are expressed as mean $\pm$ standard deviation. The homoscedacity and normality of the data sets were checked beforehand using Hartley's test. Once the conditions of normality and homoscedacity had been met, the ANOVA test was performed at the 5% significance level. Differences were considered significant at $P < 0.05$. When the conditions of normality and homoscedacity were not met, the Kruskal Wallis test was applied. The various statistical tests were performed using Past 3 software.

III. RESULTS AND DISCUSSION

III.1. Mineral characterisation of *Pennisetum purpureum* stems carbonate extracts

Analysis of the mineral constituents of *Pennisetum purpureum* stems carbonated extract reveals the presence of several minerals, in particular K, S, Rh, Fe, Cu, Br and Rb (Table II). The most abundant minerals are Potassium (K) and Sulphur (S) with proportions of 58474.32 ppm and 4786.099 ppm respectively. Bromine (Br) is the least abundant mineral with an estimated proportion of 9,512 ppm. These relatively high proportions of mineral elements in the *Pennisetum purpureum* stems carbonated extract indicate the high mineral potential of this extract.

Table II: Qualitative and quantitative minerals analysis of *Pennisetum purpureum* stems Carbonated extract

Nº	Minerals elements Names	Minerals elements quantitative contents (ppm)
1	Potassium (K)	58474.32
2	Sulfur (S)	4786.099
3	Rubidium (Rb)	82.103
4	Iron (Fe)	34.853
5	Copper (Cu)	30.507
6	Bromine (Br)	9.512

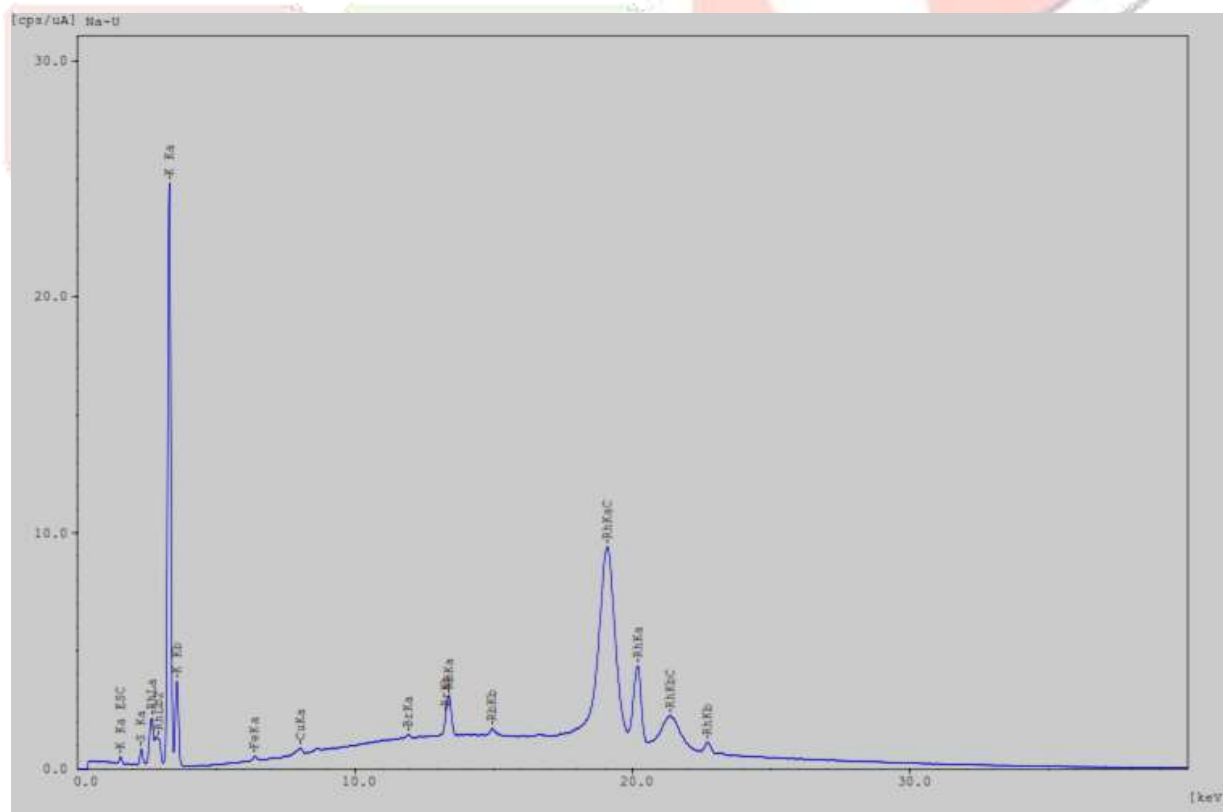


Figure 2: EDX analysis spectrum of *Pennisetum purpureum* stems carbonated extract

Notes: X= Effective Energy (keV); Y= Fluorecent X ray intensity of quantitative element (cps/uA)

III.2. Effect of experimental culture media on biomass production

A comparative analysis of the biomass from the different experimental growing media based on *Pennisetum purpureum* stems carbonated extract at doses of 300g/l; 350g/l; 400g/l; 450g/l and 500g/l respectively, compared with Jourdan's reference medium, did not reveal any significant difference ($p = 0.213 > 0.05$) between the treatments. All the treatments had a similar effect on biomass production, with a mean dry biomass value estimated at 1.36 ± 0.60 g/l, although the post-harvest biomasses from the different media varied during the different harvest periods (Figure 3).

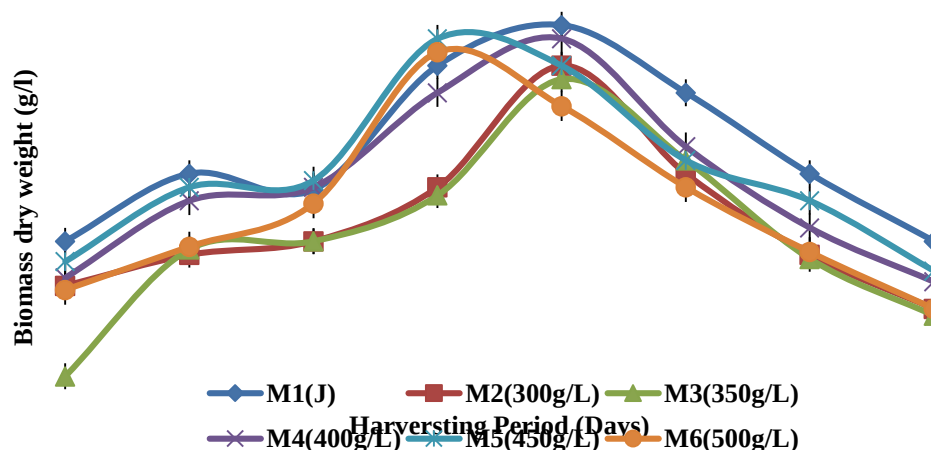


Figure 3: Changes in dry biomass production of *Artrosira platensis* grown in different experimental growing media based on *Pennisetum purpureum* stems carbonated extract compared with Jourdan's reference medium.

Notes: M1 = Modified Jourdan medium; M2 = Culture medium based on *Pennisetum purpureum* stems carbonated extract at a dose of 300g/l; M3 = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at a dose of 350g/l; M4 = Culture medium based on *Pennisetum purpureum* stems carbonated extract at 400g/l; M5 = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at 450g/l; M6 = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at 500g/l.

III.3. Effect of different experimental environments on the nutritional quality of post-harvest biomass

A comparative analysis of a number of nutritional parameters (total protein content, carbohydrate, total lipids, iron and energy value) of post-harvest biomass from different experimental growing media based on *Pennisetum purpureum* stems carbonated extract at different doses (300 g/l; 350 g/l; 400 g/l; 450 g/l; 500g/l) compared with Jourdan's reference medium shows a significant difference ($P < 0.05$) between treatments (Figure 4). A comparative analysis of the total protein content of the post-harvest biomass from the different experimental growing media reveals a significant difference ($P = 0.04 < 0.05$) between the treatments (Figure 4A). Medium M5 (450g/l) recorded the highest protein content, with an estimated average of 605.47 ± 0.0 mg/g, while the lowest was obtained from the dry biomass of medium M4 (400g/l), with an average value of 244.14 ± 0.0 mg/g. Statistical analysis of the carbohydrate content also revealed a significant difference between treatments ($p = 0.00 < 0.05$). Biomass from medium M6 (500g/l) had a significantly higher effect than that from the other treatments (Figure 4B). The average content was estimated at 683.33 ± 14.434 mg/g. Biomass from medium M2 (300g/l) obtained the lowest value, i.e. an average of 116.67 ± 14.43 mg/g. As for the total lipid content, medium M6 (500 g/l) had a significantly higher effect than the other experimental media (Figure 4C), with a mean total lipid value estimated at $7.00 \pm 0.0\%$. The lowest content was obtained from biomasses from medium M3 (350g/l), with an estimated mean value of $1.44 \pm 0.12\%$. Analysis of the energy value of biomass from the different experimental media also revealed a significant difference between treatments (Figure 4D). The biomasses from medium M6 (500 g/l) obtained a higher energy value than those from the other media, i.e. an average value of 493.82 ± 35.32 Kcal. The lowest value was recorded for biomasses from medium M4 (400g/l), with an average value of 246.34 ± 29.74 Kcal. However, the biomasses from medium M5 (450 g/l) recorded a significantly higher iron content than those from the other experimental media ($p = 0.044 < 0.05$), i.e. an average value of 0.18 ± 0.03 ppm (Figure 3E). The lowest content was obtained from biomasses from medium M4 (400g/l), i.e. an average value of 0.058 ± 0.0 ppm.

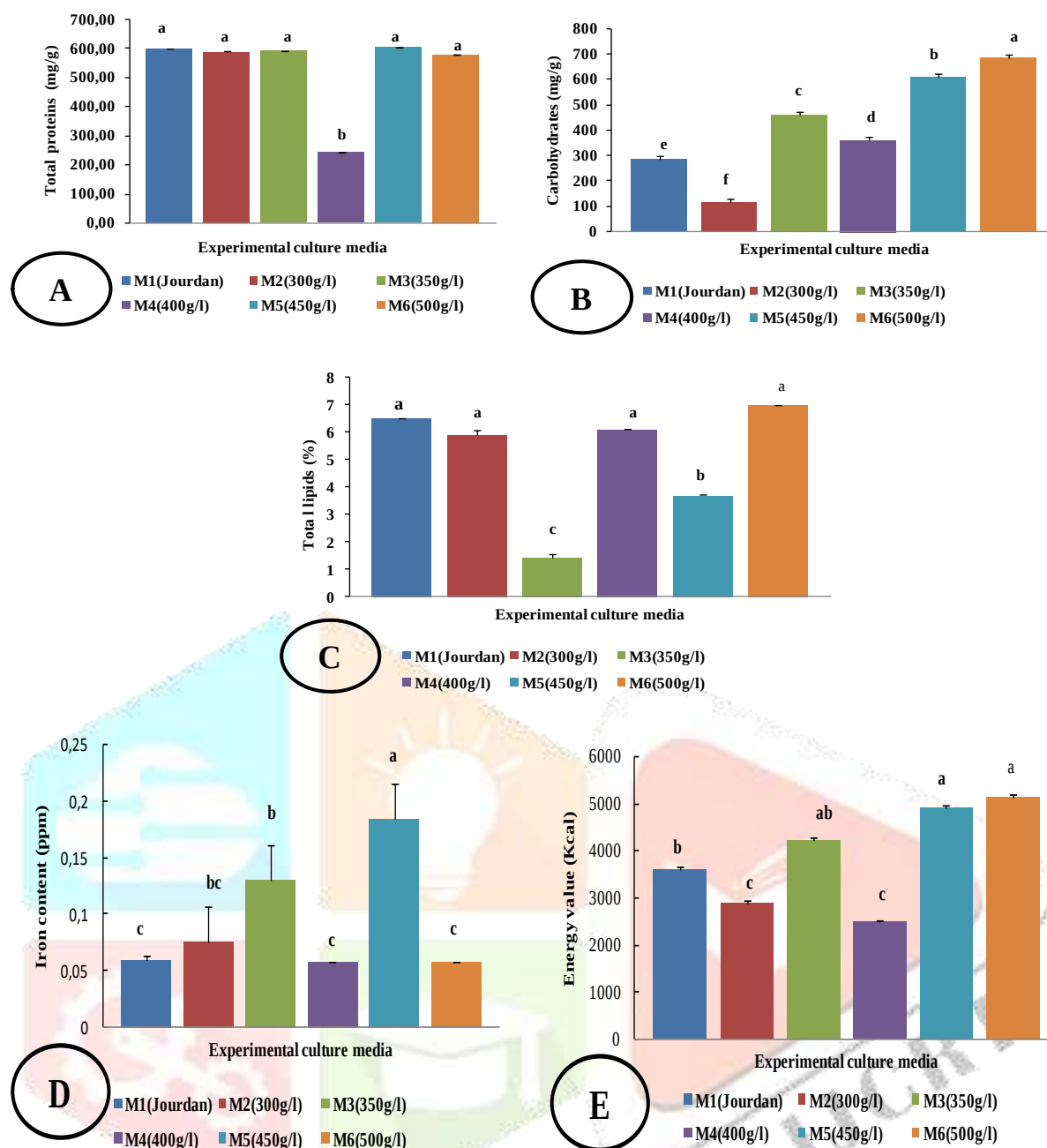


Figure 4: Variation in the total protein (A), Carbohydrates (B) total lipid (C), energy (D) and iron (E) content of post-harvest biomass from different experimental media based on *Pennisetum purpureum* stems carbonated extract compared with Jourdan's reference medium.

Notes: Vertical bars with the same letter are not significantly different ($p < 0.05$; $\bar{x}$ = standard deviation of the mean ($n=3$)). M1 = Modified Jourdan medium; M2 = Culture medium based on *Pennisetum purpureum* stems carbonated extract at a dose of 300g/l; M3 = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at a dose of 350g/l; M4 = Culture medium based on *Pennisetum purpureum* stems carbonated extract at 400g/l; M5 = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at 450g/l; M6 = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at 500g/l.

III.4. Effect of different experimental culture media on general pigment content

Analysis of the chlorophyll a and b content (Figure 5A and Figure 5B) of the post-harvest biomass from the different experimental media revealed a significant difference between the treatments ($p < 0.05$), with respective mean values of 4.9 ± 1.8 mg/g (for chlorophyll A) and 5.65 ± 1.05 mg/g (for chlorophyll B). Biomass from medium M6 (500g/l) recorded the highest levels of chlorophyll A and B, with respective averages of 7.29 ± 0.01 mg/g and 7.07 ± 0.01 mg. Biomass from medium M2 (300g/l) had the lowest levels of both Chlorophyll A and B, with averages of 2.58 ± 0.0 mg/g and 4.15 ± 0.0 mg/g respectively. Statistical analysis of carotenoid content (Figure 5C) also revealed a significant difference between treatments ($p < 0.05$). Biomass from medium M6 (500g/l) had the highest content, with an estimated mean value of 10.82 ± 0.0 mg/g, whereas biomass from medium M2 (300g/l) had the lowest content, with a mean value of 3.82 ± 0.01 mg/g. Similarly, analysis of the phycocyanin content (Figure 5D) of post-harvest biomasses shows a significantly higher effect

of medium M6 (500g/l) with an estimated mean content of 0.95 ± 0.01 mg/g, while those from medium M2 (300g/l) had the lowest content with a mean value of 0.53 ± 0.0 mg/g.

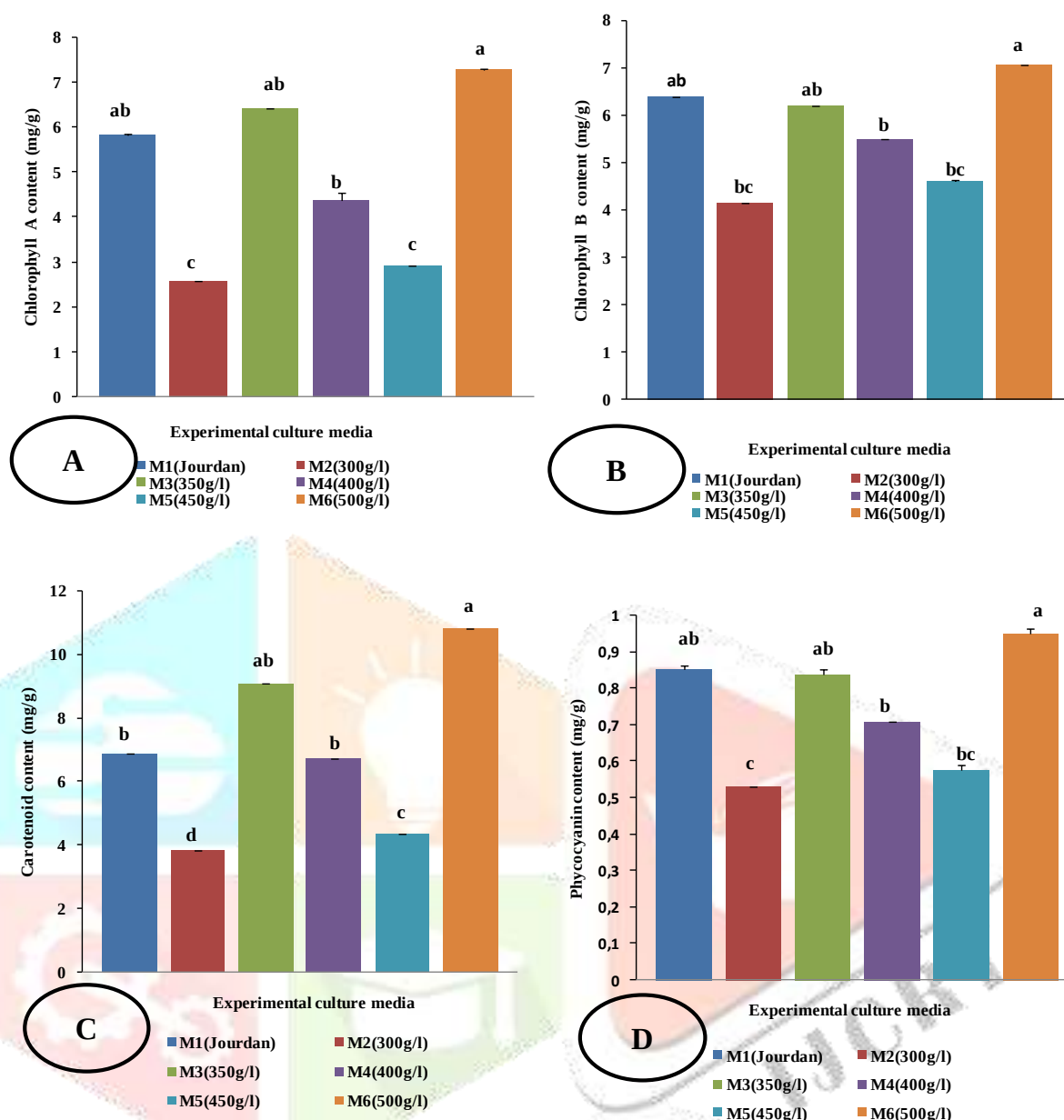


Figure 5: Variation in the Chlorophyll A (A), Chlorophyll B (B), Carotenoid (C) and Phycocyanin(D) content of post-harvest biomass from different experimental media based on *Pennisetum purpureum* stems carbonated extract compared with Jourdan's reference medium

Notes: Vertical bars with the same letter are not significantly different ($p < 0.05$; $\bar{x}$ = standard deviation of the mean ($n=3$)). M1 = Modified Jourdan medium; M2 = Culture medium based on *Pennisetum purpureum* stems carbonated extract at a dose of 300g/l; M3 = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at a dose of 350g/l; M4 = Culture medium based on *Pennisetum purpureum* stems carbonated extract at 400g/l; M5 = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at 450g/l; M6 = Culture medium based on of *Pennisetum purpureum* stems carbonated extract at 500g/l.

IV. DISCUSSION

The main physicochemical parameters measured during this experimental phase, i.e. the temperature and pH of the culture media, had average values in line with the requirements for adequate growth of *Arthrospira platensis* as reported by Jourdan (2014), i.e. average values of between 20°C and 40°C for temperature, and 8.5 to 11.5 for pH. Mean values ranged from 20°C to 40°C for temperature and 8.5 to 11.5 for pH. Temperature and pH values during the experimental period oscillated in the mean range of $26.8 \pm 1.21^\circ\text{C}$ to $26.92 \pm 1.18^\circ\text{C}$ for temperature and 9.50 ± 0.5 to 9.52 ± 0.6 for pH. This result corroborates that of Mutlen et al.(2019) who obtained mean temperatures ranging from $29.13 \pm 5.28^\circ\text{C}$ and $24.6 \pm 1.3^\circ\text{C}$. These high pH values reflect the good photosynthetic activity of the cyanobacteria in the different culture media.

According to Doumandji et al (2012), an increase in pH is a positive indicator of the photosynthetic efficiency of *Arthrospira platensis*.

Analysis of the mineral constituents of *Pennisetum purpureum* stems carbonated extract reveals the presence of several minerals, in particular K, S, Rh, Fe, Cu, Br and Rb. The most abundant minerals are potassium (K) and Sulphur (S), with proportions of 58474.32 ppm and 4786.099 ppm respectively. Bromine (Br) is the least abundant mineral, with an estimated 9,512 ppm. However, it should be noted that this extract lacks certain major mineral elements such as magnesium, calcium and phosphate. The absence of these major minerals could influence the overall mineral balance of the experimental culture media based on this extract and therefore impact production. Despite this difference in mineral content, it should be noted that the proportions of certain minerals are in line with the minimum values recommended by Jourdan for the preparation of a new nutrient medium for *Spirulina* culture. These values concern potassium (642 mg/l) and iron (1 mg/l).

A comparative analysis of the biomass from the different experimental growing media based on *Pennisetum purpureum* stems carbonated extract at doses of 300g/l; 350g/l; 400g/l; 450g/l and 500g/l respectively, compared with Jourdan's reference medium, did not reveal any significant difference ($p=0.213 > 0.05$) between the treatments. All the treatments had a similar effect on biomass production, with a mean dry biomass value estimated at 1.36 ± 0.60 g/l. This result shows that the dose of *Pennisetum purpureum* stems carbonated extract does not significantly influence biomass production. These results are lower than the observations of Mutlen et al. (2019), who obtained average biomass production ranging from 4.88 ± 0.11 g/l to 4.99 ± 0.21 g/l. This difference could be explained by the use of different culture media. This difference could be explained by the use of different culture media. The *Pennisetum purpureum* stems carbonated extract was used to prepare the semi-biological culture media in our experiments, whereas *Laportea aestuans* and *Manihot esculenta* raw extracts were used by these authors to prepare the experimental culture substrates. This difference in terms of culture substrate could explain the difference in the growth of *Arthrospira platensis* in these media.

A comparative analysis of many nutritional parameters (total protein content, carbohydrate, total lipids, iron and energy value) of post-harvest biomasses from different experimental growing media based on *Pennisetum purpureum* stems carbonated extract at different doses (300 g/l; 350 g/l; 400 g/l; 450 g/l; 500g/l) compared with Jourdan's reference medium shows a significant difference ($P < 0.05$) between treatments. Concerning the average protein content of the post-harvest biomasses from the different experimental growing media, the average values ranged from 244.14 ± 0.0 mg/g to 605.47 ± 0.0 mg/g. Medium M5 (450g/l) recorded the highest protein content, with an estimated average of 605.47 ± 0.0 mg/g. However, these results are lower than those obtained by Mutlen et al (2019), with an average value of $55.44 \pm 0.38\%$. This difference could be explained by the difference in growing substrates used, the harvesting time and even the drying technique as reported by Mohamed (2011). Analysis of carbohydrate content shows differences between the different culture media with mean values ranging from 683.33 ± 14.434 mg/g to 116.67 ± 14.43 mg/g. Medium M6 (500g/l) recorded the highest value. However, only the biomasses from media M1 (Jourdan) and M2 (300g/l) had carbohydrate contents within the range of the *Spirulina* carbohydrate content as stipulated by Cruchot (2008), i.e. from 15 to 25% with a relative margin of 10%. Biomass from other culture media, on the other hand, had average values outside this range. This high variability in carbohydrate content could be associated with the dose of extract, which would significantly influence the availability of this constituent in the post-harvest biomass. As for the total lipid content, the mean values ranged from $7.00 \pm 0.0\%$ to $1.44 \pm 0.12\%$. The M6 medium (500 g/l) had a significantly higher effect than the other experimental media, with a mean total lipid value estimated at $7.00 \pm 0.0\%$. In addition to the relatively low total lipid values recorded in culture media M3 (350g/l) and M5 (450g/l), those recorded for the biomasses from the other culture media (M1(J); M2(300g/l); M4(400g/l); M6(500g/l)) were in line with the values recommended by Xue (2002) and Cohen (1997), i.e. values of between 6 and 13% of the dry weight of *Spirulina*. Analysis of the energy value of the post-harvest biomasses reveals a significantly higher effect of the M6 medium (500 g/l) compared with the other experimental media, with an estimated mean value of 493.82 ± 35.32 Kcal. However, the average energy value of the biomass from the different experimental media, 386.01 ± 33.22 mg/g, was still higher than those recorded by Derdouri et al (2021) and Dansou (2002), with average values of 327.02 Kcal and 338 Kcal respectively. This difference is related to the higher protein, lipid and carbohydrate contents in the biomasses from our experimental culture media compared with those from these authors' samples. Concerning iron content, the biomasses from medium M5 (450 g/l) had a significantly higher iron content than those from the other experimental media ($p = 0.044 < 0.05$), i.e. an average value of 0.18 ± 0.03 ppm (Figure 3E). The lowest content was obtained from biomasses from medium M4 (400g/l), i.e. an average value of 0.058 ± 0.0 ppm.

An analysis of the general pigments in the post-harvest biomass from the different experimental media revealed a significant difference between the treatments ($p < 0.05$) for chlorophyll A and B content, with respective mean values of $4.9 \pm 1.8 \text{ mg/g}$ (for chlorophyll A) and $5.65 \pm 1.05 \text{ mg/g}$ (for chlorophyll B). Biomass from medium M6 (500g/l) recorded the highest levels of both chlorophyll A and B, with respective averages of $7.29 \pm 0.01 \text{ mg/g}$ and $7.07 \pm 0.01 \text{ mg/g}$. These results suggest that the dose of extract influences the chlorophyll content of post-harvest biomass. The chlorophyll contents obtained are higher than those reported by Manet (2016), whose average was estimated at 60 mg/10g . This difference could be associated with the culture substrate, but also with the time at which the biomass was harvested, which would influence the availability of this pigment in the samples. Analysis of carotenoid content also revealed a significant difference between treatments ($p < 0.05$). Biomass from medium M6 (500g/l) recorded the highest content with an estimated mean value of $10.82 \pm 0.0 \text{ mg/g}$ while biomass from medium M2 (300g/l) obtained the lowest content with a mean value of $3.82 \pm 0.01 \text{ mg/g}$. These average values are higher than those reported by Manet (2016), i.e. values between $15\text{--}24 \text{ mg/10g}$. This higher proportion of these constituents in the post-harvest biomass could be associated with the different growing substrates, which have a different effect on the availability of this constituent. As for the phycocyanin content, medium M6 (500g/l) had a significantly higher effect than the other treatments, with an estimated mean content of $0.95 \pm 0.01 \text{ mg/g}$, whereas medium M2 (300g/l) had the lowest content, with a mean content of $0.53 \pm 0.0 \text{ mg/g}$. These average phycocyanin values obtained are lower than those recommended by Falquet (2006), i.e. a content of over 15 mg/g . This low phycocyanin content in post-harvest biomass could be associated with the harvesting period and the drying technique, which could alter this component present in the harvested biomass.

V. CONCLUSION

The results of this study highlighted the mineral potential of *Pennisetum purpureum* stems carbonated extract and its potential use as an input in the preparation of new growing substrates for *Arthrospira platensis*. Analysis of the mineral constituents of *Pennisetum purpureum* stems carbonated extract reveals the presence of several minerals, notably K, S, Rh, Fe, Cu, Br and Rb. The most abundant minerals are Potassium (K) and Sulphur (S) with proportions of 58474.32 ppm and 4786.099 ppm respectively. Bromine (Br) is the least abundant mineral with an estimated proportion of $9,512 \text{ ppm}$. These results show that the dose of *Pennisetum purpureum* stems carbonate extract does not significantly influence biomass production. However, analysis of the nutritional profile of the post-harvest biomass from the different experimental media revealed a significantly higher effect of the culture medium at a dose of 500 g/l (M6) of *Pennisetum purpureum* stems carbonated extract compared with the other culture media for both carbohydrate and total lipid content. The respective mean values were $683.33 \pm 14.434 \text{ mg/g}$ (carbohydrates) and $9.06 \pm 0.05\%$ (total lipids). These higher proportions of carbohydrates and total lipids in the biomasses from this M6 culture medium (500 g/l) could justify their higher energy value (i.e. an average of $493.82 \pm 35.32 \text{ Kcal}$) compared with those obtained from biomasses from other culture media. However, analysis of protein and iron content showed a significantly higher effect of the M5 medium (450 g/l) compared with the other experimental media, with respective mean values of $605.47 \pm 0.0 \text{ mg/g}$ (total protein) and $0.18 \pm 0.03 \text{ ppm}$ (iron content). An analysis of the general pigments in the post-harvest biomass from the different experimental media revealed a significantly higher effect of the culture medium at a dose of 500 g/l (M6) of *Pennisetum purpureum* stems carbonated extract compared with the other culture media in terms of both chlorophyll A and B, carotenoids and phycocyanine content. With respective mean values of $4.9 \pm 1.8 \text{ mg/g}$ (for chlorophyll A), $5.65 \pm 1.05 \text{ mg/g}$ (for chlorophyll B); $10.82 \pm 0.0 \text{ mg/g}$ (for carotenoids) and $0.95 \pm 0.01 \text{ mg/g}$ (for phycocyanin).

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VIII. Conflicts of interest

The authors declare no conflicts of interest.

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