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Effect Of Semi-Organic Culture Media Based On *Musa paradisiaca* L. Peels Crude Extract On Growth, Soluble Protein Content, General Pigments And Secondary Metabolites Quality Of *Arthrospira platensis*.

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Abstract

Background:*The cyanobacterium Arthrospira platensis is a food source of high nutritional quality thanks to the diversity and richness of its constituents. However, its production in a synthetic medium is very costly given the excessively high mineral element requirements of this micro-alga. The aim of this study was to evaluate the effect of Musa paradisiaca peels crude extract on the growth, soluble protein content, general pigments and secondary metabolites quality of Arthrospira platensis.*

Materiels and Methods: *6 experimental culture substrates were developed, including 5 based on Musa paradisiaca peels crude extract at different doses (10g/l 20g/l, 30g/l, 40g/l, 50g/l) and 1 reference medium (modified Jourdan medium). All the media previously prepared were subsequently seeded with 5 ml / l of a strain of Arthrospira platensis for each respective medium. A daily check of the physicochemical parameters was carried out, as well as weekly harvests in order to evaluate the productivity of each culture medium. A quantitative characterization of soluble proteins, general pigments and secondary metabolites of post harvest biomasses from different media was made.*

Results*: The results show that Musa paradisiaca peels crude extract contains several major minerals, including calcium (Ca), magnesium (Mg), potassium (K), sodium (Na) and phosphorus (P). The analysis reveals a higher proportion of magnesium and calcium, with mean values of 390.333 ± 0.44 mg/l and 224 ± 0.66 m g/l respectively. Potassium and sodium, on the other hand, were relatively low, with mean values of 14.03±0.04 mg/l and 17.36±0.23 mg/l respectively. Analysis of biomass production revealed similar growth in all the experimental media, with a mean dry biomass value estimated at 1.91±0.64 g/0.5l. The same applies to the soluble protein content of the post-harvest biomass, with an average value of 175.58±60.03 mg/g. Comparative analysis of general pigments in post-harvest biomass revealed a similar effect of the different treatments (p>0.05) on chlorophyll a (28.53±2.37 µg/g) and b (16.58±7.93 µg/g) content. However, analysis of carotenoid and phycocyanin content revealed a significant difference between treatments (P= 0.008; P = 0.014), with mean values of 29.81± 0.37 µg/g and 1.20 ± 0.24 µg/g respectively. Quantitative analysis of some secondary metabolites (alkaloids, flavonoids, total phenols, tannins) in post-harvest biomass showed a significant effect of the culture medium on flavonoid content (P=0.009), with a mean value of 67±7.38 mg/g. However, the culture medium did not influence the availability of alkaloids, total phenols and tannins in the biomass, with mean values of 7.72±0.14 mg/g, 5.95±3.28 mg/g and 196±32.07 mg/g respectively.*

Conclusion*: The results of this study highlighted the mineral potential of Musa paradisiaca peels crude extract and their potential use as an input in the preparation of new growing substrates for Arthrospira platensis.*

Keywords: *Arthrospira platensis, Musa paradisiaca, growth, soluble proteins, general pigments, secondary metabolites*

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I. Introduction

The cyanobacterium *Arthrospira platensis*, also known as *Spirulina platensis*, is a photoautotrophic filamentous microalga that has been 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 now, 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 colour. 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 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 colored egg yolk, as well as the pigmentation of meat and skin that appeal to consumers (Durand-chastel, 1980 and Richmond, 1988). Phycocyanins, on the other hand, are an important source of blue pigment for use in food coloring, and are found in Spirulina in concentrations 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-djilali, 2012). 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, in view of their mineral potential. To this end, *Musa paradisiaca* L(Musaceae) peels crude exttract are interesting opportunities to explore.

Conventionally call plantain, *Musa paradisiaca* Linn. (Musaceae) is a large mono- herbaceous perennial crop (up to 9 m of long) native to Southeast Asia, grown in the tropical and subtropical world region (Strosse *et al.,* 2006). It is one of the most important food crops widely produced and consumed in sub-Saharan Africa (Nafack *et al*., 2023). The plant is tall with a sturdy pseudo stem and large broad leaves arranged spirally at the top (Egbuonu, 2016). The leaves have large blades with a pronounced central midrib and obvious veins (Egbuonu, 2016). Each pseudo stem produces a group of flowers from which the fruits develop in a hanging cluster. In Cameroon, where production is about 3 million tons per year (Nafack *et al*., 2023), plantain occupies a predominant place in households and plays an important role in the food security of the population (Nafack *et al*., 2023). Plantain is rich in phytonutrients hence has nutritional value (Happi *et al*., 2011; Onwuka *et al.*, 1997). Regarding the nutrient content, it has been reported that 100 g of plantain contain about 35.5 g of carbohydrates, 1.3 g of protein, 0.3 g of fat and 5.8 g of fiber and provide 122 kcal (Assemand, 2012). In addition, mature plantain contains many bioactive molecules, including carotenoids (4680 µg/100 g), vitamin C (11.7 mg/100 g), minerals, and phenolic compounds (Nafack *et al*., 2023). Despite the numerous uses of the various parts (leaves, inflorescence, fruits, mucilage, pseudo-stem, roots etc) of *Musa paradisiaca* L. (plantain) in traditional medicine, less has been reported about the utility of its unripe fruit peels in animal nutrition and health. The peels could be good sources of bioactive compounds but as major waste products of various fruits are essentially discarded (Egbuonu et al., 2017). Plantain peels have been reported to be very rich in phosphorus and nitrogen (Oyeyinka and Afolayan, 2019). Egbuonu, *et al*., (2017) reported that 100g of plantain peels contain 10.00 ± 0.95 mg of Calcium, 7.60 ± 0.55 mg of Magnesium, 16.20 ± 1.35 mg of Sodium, 23.50 ± 1.54 mg of Potassium, 36.00 ± 1.65mg of Phosphorus and 5.60 ± 0.06 mg of Iron. However, the use of extracts from

Musa paradisiaca 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 *Musa paradisiaca* peels crude extract on the growth, soluble protein, general pigments and secondary metabolites quality of *Arthrospira platensis*.

II. Materials And Methods

Experimental site

The study took place in the locality of Yabassi, 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.

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 ($NaCaCO_3$), 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.

***Musa paradisiaca* peels crude extract preparation**

5 kg of unripe plantain (*Musa paradisiaca*) peels were collected from households in the locality of Yabassi. Once harvested, they were washed and drained. At the end of this phase, the peels were crushed using a mortar and then pressed by hand. The product obtained from this process was filtered on a fine cloth and 3 liters of crude extract was obtained.

Quantification of some major mineral constituents of *Musa paradisiaca* peels crude extract

The main major mineral constituents quantified in the crude extract were calcium, magnesium, potassium, sodium and phosphorus. The choice of these mineral elements was based on the substitution of certain synthetic inputs in the culture media by *Musa paradisica* peels crude extract. The synthetic inputs substituted were potassium nitrate, magnesium sulphate, calcium chloride.

The sample for the determination of the elements was subjected to acid digestion using concentrated hydrochloric acid and subsequently, the different elements were determined using appropriate methods as described by James (2005). 20 gm of each sample was burnt to ashes in a muffle (as in ash determination). The resulting ash was dissolved in HCl (1 mL HCl) and then diluted to 100 mL in a volumetric flask using distilled water. The digest obtained was used for various analyses.

Determination of Calcium and Magnesium

Calcium and magnesium contents of the digested sample were determined by complexiometric titration. 10 mL of the sample was dispensed into a separate conical flask. Pinch of the masking agents, potassium cyanide, potassium ferrocyanide and hydroxyl hydrochloride were measured into the content of each flask. 20 mL of ammonia buffer was added to one of the flasks to raise the pH to 10.0, while 10 mL of NaOH solution was added to the other to raise the pH to 12.0. To the flask at pH 10 (for cacium and magnesium) Erichrome dark black indicator was added and titrated against 0.02 N EDTA solution and the other black was added and titrated against 0.02 N EDTA solution at pH 12.0 (for calcium alone). Selechrome dark blue indicator was added and titrated against 0.02 N EDTA solution at pH 12.0. Calcium form complexes with EDTA at pH 12.0, while both calcium and magnesium form complexes with EDTA at pH 10.0. A reagent blank was titrated as a control. The calcium and magnesium content of the samples were calculated using the standard that 1mL of 1 N EDTA has an equivalence of 24mg magnesium and 20.04 mg calcium.

Determination of Sodium and Potassium

Sodium and potassium were determined by flame photometry method. 1 mL of the prepared potassium and sodium standard were aspirated into the machine and sprayed over the non-luminous butane gas flame. The sodium and potassium emission (having been appropriately filtered) from the different concentration were

recorded and made into standard curve. Subsequently, the optimal density emission was recorded from each of the sample against those in the curve. Thus using the curve was used to extrapolate the quantity of each (sodium and potassium) in the sample.

Determination of Phosphorus

The phosphorus in the sample was determined by the Vanado-molybdate (yellow) spectrometry. 1 mL extract from the sample was dispensed into the test tube. Similarly, the volume of the standard phosphorus solution as well as water was put into another test tube to serve as standard and blank respectively. The content of each tube were mixed with equal volume of Vanado-molybdate colour reagent. They were left to stand for 15 min at room temperature before their absorbance was measured in Jenway electronic spectrophotometer at wavelength of 420 nm. Measurements were taken with the blank at zero. Phosphorus content was given by the formula: mg/100gm = 100 W x Au As x C x Vf Va Where: W= Weight of sample analyzed Au= Absorbance of the test sample As= Absorbance of standard solution Vf= Total volume of filtrate Va= Volume of filtrate analyzed C= Concentration of the standard.

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.

Culture media (g/l)						
Inputs	**M_1**	**M_2**	**M_3**	**M_4**	**M_5**	**M_6**
Sodium bicarbonate ($NaCaCO_3$)	08	-	-	-	-	-
Trisodium phosphate ($P2O_5$)	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	0,02	0,02	0,02	0,02	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
***Musa paradisiacal* peels crude extract**	-	10	20	30	40	50

Notes: M1 = Modified Jourdan medium; M2 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 10g/l; M3 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 20g/l; M4 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 30g/l; M5 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 40g/l; M6 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 50g/l.

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 m^2 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.

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 (35ºC on average) and then reweighed. The dry weight was evaluated in g.l-1(AOAC, 2000).

Quantification of general pigments, soluble proteins and secondary metabolites in post-harvest biomasses

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

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).

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.

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 x (OD 615- 0.473 x OD 652) x 100] / C; C being the concentration of Spirulina in the solution.

Quantification of soluble proteins

Quantification of soluble proteins was carried out by absorption spectrophotometry using the method described by Warburg and Christian (1941). The quantity of protein was measured by Absorption spectrophotometry at 280 nm with a maximum around 254 nm. Absorption is measured at 280 nm to perform a quantitative or semi-quantitative assay of a protein solution. A solution containing 1 mg of protein/mL has an A280 of the order of 0.5 to 2.0. For a pure protein, the absorption coefficient can be determined empirically. [Protein](mg /mL) = 1.55 A280 - 0.76 A260

Quantification of secondary metabolites

Alkaloids contents

The assay was performed using the spectrophotometric method described by Sreevidya N. and Mehrotra S (2003). 5mL of extract solution was prepared and the pH was maintained between 2 and 2.5 with dilute HCl. 2mL of Dragendorff's reagent was added to the preparation and the precipitate formed was centrifuged. The centrifugate was checked for complete precipitation by adding Dragendorff's reagent and the centrifuged mixture was decanted completely. The precipitate was washed with alcohol. The filtrate was discarded and the residue was then treated with 2 ml of di-sodium sulphate solution. The brownish-black precipitate formed was then centrifuged. Completion of precipitation was checked by adding 2 drops of disodium sulphate. The residue was dissolved in 2mL of concentrated nitric acid, warming if necessary. This solution was diluted to 10mL with distilled water. Then 1mL of this diluted solution was taken and 5mL of thiourea solution was added. The absorbance was measured at 435 nm. The standard curve was made from a stock solution of atropine at 10mg/L with a range from 0 to 1mg/mL. The absorbances were read using a spectrophotometer at 435 nm against the white tube prepared under the same conditions by replacing the sample with distilled water. The alkaloid content of the samples was estimated from the linear regression line and expressed in gram equivalents of atropine per 100g of powder.

Total flavonoids content

The determination of total flavonoid content was done by the method described by Arvouet – Grand *et al*. in 1994. A volume of aluminum chloride in methanol (2 mL, 2%) was mixed with the methanolic solution of

the extract (2 mL, 1mg/mL). After 10 min incubation, the measurements of absorbances were done at 415 nm. The calibration curve equation was used to determine the total flavonoid content. The previous formula used for the total phenolic content is the same used here and expressed in mg of Quercetin Equivalents (QE)/100g dry material (Miliauskas *et al*., 2004). All measurements were duplicated.

Total phenolics content

The determination of total phenolic content was performed according to the Folin-Ciocalteu method (Mahamane *et al*., 2020; Kouadio *et al*., 2021). In fact, 0.5 mL of the diluted extract (1 mg/mL) was mixed to 2.5 mL of the reagent of dilute Folin-Ciocalteu 0.2 N (1:10 dilution) and then shaken and left to stand for five minutes at room temperature to allow for the reagent to react completely with the oxidible substances or phenolates. 2 mL of Na_2CO_3 (5% in water) were added to destroy the residual reagent. The measurements of absorbances were performed with a spectrophotometer at 760 nm, after incubation for 2 hours in darkness against a blank (distilled water). The calibration curve equation was used to determine the total phenolic contents of the samples. They were expressed in mg Gallic Acid Equivalents (GAE)/100 g of dry material (Singleton *et al.*, 1999). All measurements were duplicated. $\boldsymbol{C = C1 \times V\, m}$ Where C expressed in mg equivalent gallic acid/g of dry material is the content of total polyphenols, C1 expressed in mg/L is the concentration of gallic acid derived from the calibration curve, V expressed in L, is the volume of extract and m expressed in g, is the weight of the plant extract (Mahamane *et al*., 2020; Kouadio *et al*., 2021).

Total tannins content

Tannin was determined using the reaction described by BateSmith (Bate-Smith & Swain, 1967). In fact, 2 mL of each plant extract (1mg/mL) was introduced in a hydrolysis tube and 3 mL of hydrochloric acid (37%) was added. The closed glass tube was heated at 100°C in a water bath for 30 minutes after which the measurement of the optical density was done at 550 nm. The control was done with the same solution kept in a tube and left at room temperature. The following formula allowed to calculate the total tannin contents: C = 19.33 (Doh – Dot), where C expressed in g/L is the total tannin content, Doh is the optical density of the hydrolyzed tube and Dot is the optical density of the control tube (Ribereau-Gayon , 1966; Hamadou *et al*., 2018; Mahamane *et al*., 2020; Kouadio *et al*., 2021) .

Statistical analysis

Results are expressed as mean ± 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 Statistic v.10software.

III. Results

Quantification of some major mineral constituents of *Musa paradisiaca* peels crude extract

Quantitative analysis of some major mineral constituents (Calcium (Ca), Magnesium (Mg), Potassium (K), Sodium (Na) and Phosphorus (P)) in the crude extract of *Musa paradisiaca* peels reveals a higher proportion of Magnesium and Calcium with mean values of 390.333±0.44 mg/l and 224±0.66 mg/l respectively. Potassium and sodium, on the other hand, have relatively low proportions, with mean concentrations of 14.03±0.04 mg/l and 17.36±0.23 mg/l respectively. These relatively high proportions of major mineral elements in the carbonate extract of *Musa paradisiaca* indicate the high mineral potential of this extract.

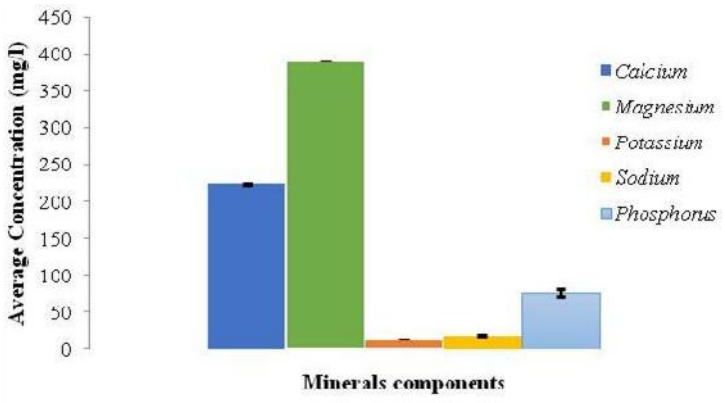

Figure 1: Quantitative Minerals analyses of *Musa paradisiaca* peels crude extract

Effect of different experimental growing media on biomass production

A comparative analysis of the dry biomass produced by the different experimental growing media based of *Musa paradisiaca* peels crude extract at respective doses of 10g/l; 20g/l; 30g/l; 40g/l; 50g/l compared with Jourdan's reference medium did not reveal any significant difference ($p > 0.05$) between the treatments (Figure 2). In fact, all the treatments had a similar effect on biomass production, with a mean dry biomass value estimated at 1.91±0.64 g/0.5l, although the post-harvest biomasses from the different media varied during the different harvest periods (Figure 2).

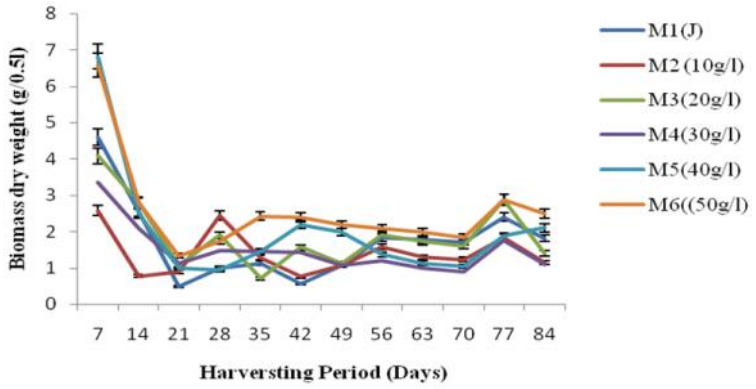

Figure 2: Evolution of dry biomass production of *Artrospira platensis* in the different experimental media based on *Musa paradisiaca* peels crude extract compared with Jourdan's reference medium during the experimental period.

Notes: M1 = Modified Jourdan medium; M2 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 10g/l; M3 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 20g/l; M4 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 30g/l; M5 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 40g/l; M6 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 50g/l.

Effect of experimental media on soluble protein content of post-harvest biomass

A comparative analysis of the soluble protein content of the post-harvest biomass from the different experimental growing media based on *Musa paradisiaca* peel crude extract compared with Jourdan's reference medium showed no significant difference ($P = 0.74 > 0.05$) between the treatments. In fact, all the treatments had a similar effect on the soluble protein content of the post-harvest biomass, with a mean value estimated at 175.58 ± 60.03 mg/g, although the post-harvest biomass from the different media varied during the different harvest periods (Figure 3).

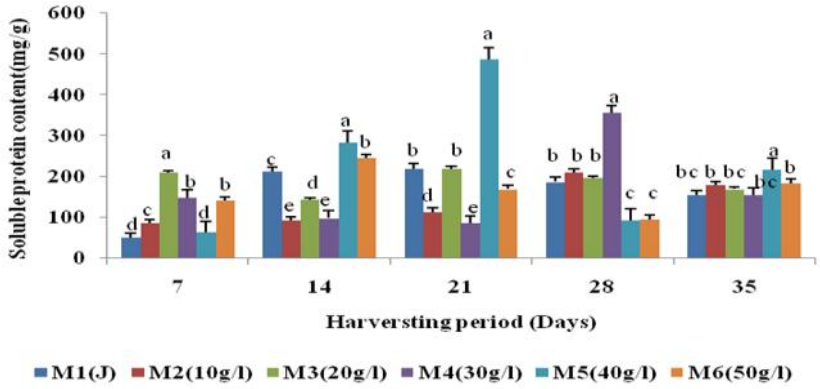

Figure 3: Variation in the soluble protein content of post-harvest biomass from different experimental media based on *Musa paradisiaca* peel crude extract compared with Jourdan's reference medium during the first 5 weeks of experimentation.

Notes: Vertical bars with the same letter are not significantly different ($p<0.05$; ┬ = standard deviation of the mean (n=3)). M1 = Modified Jourdan medium; M2 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 10g/l; M3 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 20g/l; M4 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 30g/l; M5 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 40g/l; M6 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 50g/l.

Effect of different experimental culture media on general pigment content

Analysis of the chlorophyll a and b content (Figure 4A and Figure 4B) of the post-harvest biomass from the different experimental environments shows a similar effect between treatments (P >0.05), with respective mean values of 28.53±2.37 µg/g (for chlorophyll a) and 16.58±7.93 µg/g (for chlorophyll b). However, analysis of carotenoid content (Figure 4C) revealed a significant difference between treatments (P = 0.008< 0.05). Biomass from Jourdan's Réferentiel medium had the highest carotenoid content, with a mean value of 30.66±0.08µg/g, while the lowest was recorded in biomass from the 20g/l *Musa paradisiaca* crude extract medium, with a mean value of 25.97±3.87µg/g. An analysis of the correlation between harvesting time and carotenoid content revealed a positive correlation between these parameters (R2 = 0.68; y = -14.812+207.86). Similarly, analysis of the phycocyanin content (Figure 4D) of post-harvest biomass showed a significant difference between treatments (P= 0.014<0.05). Biomass from the medium containing 50g/l of *Musa paradisiaca* crude extract had the highest value, i.e. an average of 1.20±0.24µg/g, whereas biomass from the medium containing 30g/l of *Musa paradisiaca* crude extract had the lowest value, i.e. an average of 0.63±0.06µg/g. An analysis of the correlation between harvesting period and phycocyanin content revealed a positive correlation between these parameters (R2=0.68; y = -14.812+207.86)

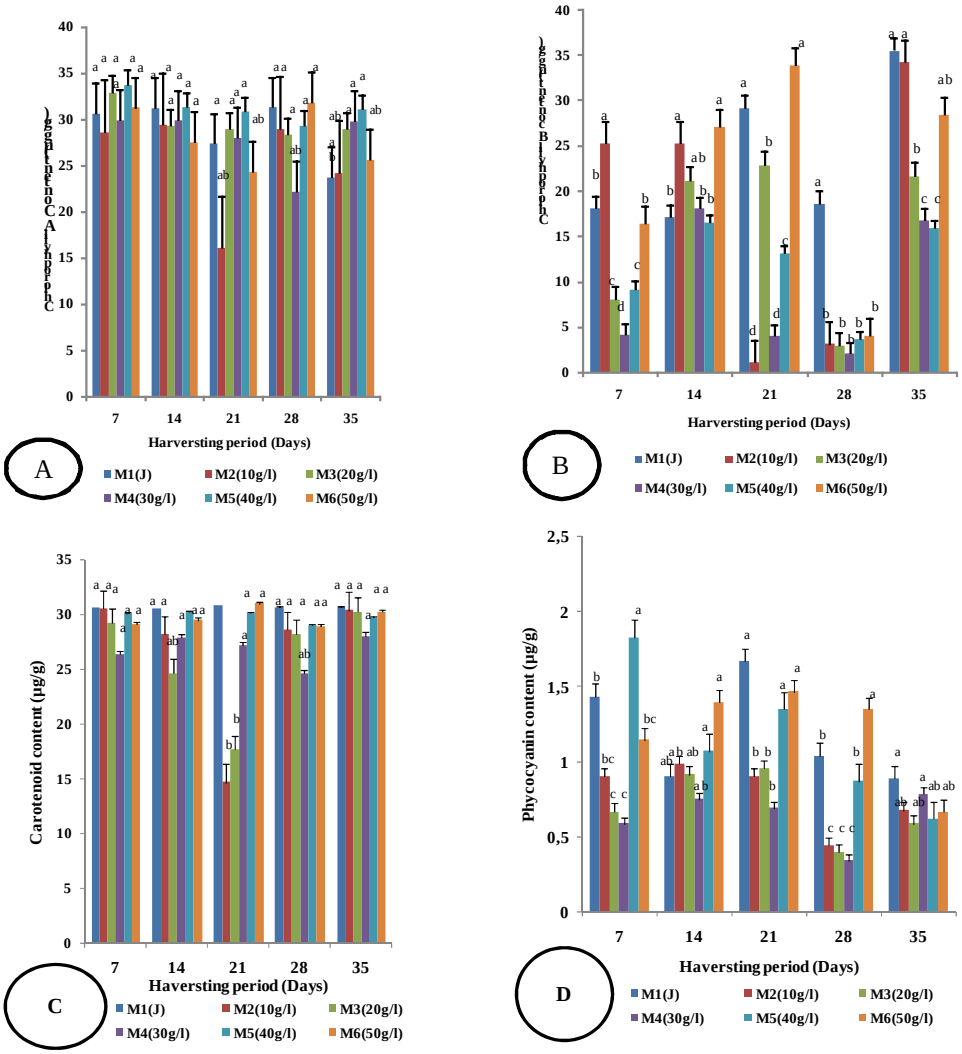

Figure 4: Variation in the Chlorophyll A (A), Chlorophyll B (B), Carotenoid (C) and Phycocyanin(D) content of post-harvest biomass from different experimental media based on *Musa paradisiaca* peel crude extract compared with Jourdan's reference medium during the first 5 weeks of experimentation.

Notes: Vertical bars with the same letter are not significantly different (p<0.05; ⊤ = standard deviation of the mean (n=3)). M1 = Modified Jourdan medium; M2 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 10g/l; M3 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 20g/l; M4 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 30g/l; M5 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 40g/l; M6 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 50g/l.

Effect of different experimental culture media on the quality of secondary metabolites in post-harvest biomass

Quantitative analysis of a number of secondary metabolites (alkaloids, flavonoids, total phenols, tannins) in post-harvest biomass from the experimental growing media shows a variation in the content of these compounds during the experimental phase (Figure 5). However, statistical analysis did not reveal any significant difference between treatments in terms of alkaloids (P=0.20 > 0.05), tannins (P=0.33 > 0.05) and total phenols (P= 0.47> 0.05). With mean values of 7.72±0.14 mg/g (for alkaloids), 5.95 ± 3.28 mg/g (for tannins), and 196±32.07 mg/g (for total phenols), respectively. However, analysis of the flavonoid content of post-harvest biomass revealed a significant difference between treatments (P = 0.009 < 0.05). The highest flavonoid content was obtained in the biomasses from the Jourdan reference medium, with an average value of 67±7.38 mg/g, while the lowest was recorded in the biomasses from the medium containing 30g/l of *Musa paradisiaca* crude extract, with an average value of 47.02±1.16 mg/g. On the other hand, media containing 10g/l, 20g/l, 40g/l and 50g/l of *Musa paradisiaca* crude extract had a similar effect, with respective values of 50.85±7.05 mg/g (M2=10g/l), 56.45±9.62 mg/g (M3=20g/l), 64.89±13.11 mg/g (M5=40g/l) and 62.34±9.07mg/g (M6=50g/l). An analysis of the correlation between harvest period and flavonoid content revealed a positive and significant correlation between these parameters (R2= 0.68; y = -14.812+207.86).

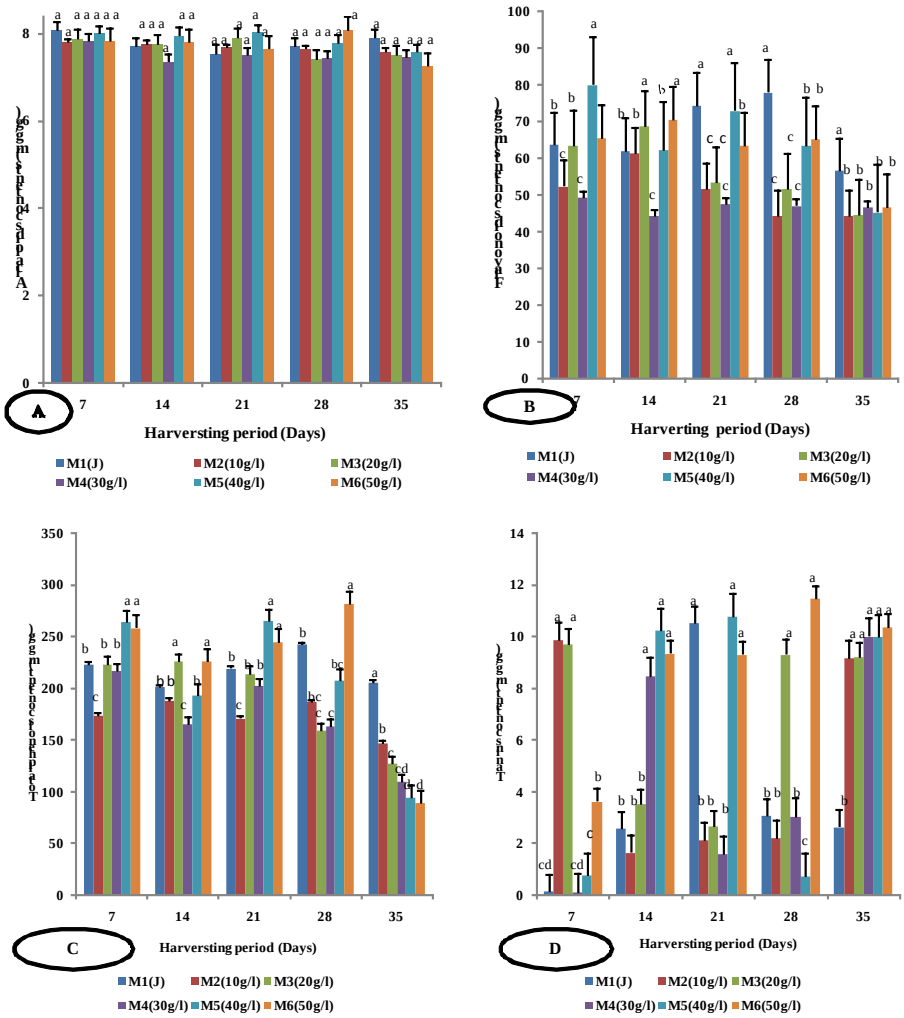

Figure 5: Variation in the Alcaloids (A), Favonoids (B), Phenols(C) and Tanins(D) content of post-harvest biomass from different experimental media based on *Musa paradisiaca* peel crude extract compared with Jourdan's reference medium during the first 5 weeks of experimentation.

Notes: Vertical bars with the same letter are not significantly different (p<0.05; ┬ = standard deviation of the mean (n=3)). M1 = Modified Jourdan medium; M2 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 10g/l; M3 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 20g/l; M4 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 30g/l; M5 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 40g/l; M6 = Culture medium based on *Musa paradisiaca* peels crude extract at a dose of 50g/l.

IV. Discussion

The main physico-chemical 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 *Artrospira 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 were between 20°C and 40°C for temperature, and 8.5 to 11.5 for pH. In fact, temperature and pH values during the experimental period oscillated in the mean range of 27.92 ±1.61°C to 28.14±1.48°C for temperature and 10.09±0.68 to 10.13 ± 0.70 for pH. This result corroborates that of Mutlen *et al*.(2019) who obtained mean temperatures ranging from 29.13±5.28°C and 24.6±1.3°C. These high pH values reflect 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 some of the major mineral constituents of *Musa paradisiaca* peels crude extract reveals a higher proportion of magnesium and calcium, with mean values of 390.333 ± 0.44 mg/l and 224±0.66 mg/l respectively. On the other hand, the proportions of potassium and sodium are relatively low, with mean concentrations of 14.03 ± 0.04 mg/l and 17.36 ± 0.23 mg/l respectively. These proportions of potassium and sodium are lower than those obtained by Egbuonu et al (2017), with mean values of 235.0 ± 1.54 mg/l of potassium and 162.0 ± 1.35 mg/l of sodium, while those of magnesium and calcium are higher than those obtained by the same authors (76.0 ± 0.55 mg/l of magnesium and 100.0 ± 0.95 mg/l of calcium). This difference in mineral elements proportions could be associated with the difference in the strain of *Musa paradisiaca*, the time of harvest, but also the nature of the soil of the geographical area in which the plant developed. All that's factors could influence the distribution and content of minerals in all parts of the plant. 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 development of a new nutrient medium for spirulina cultivation.

A comparative analysis of the dry biomass from the different experimental media based on *Musa paradisiaca* peels crude extract compared with Jourdan's reference medium did not reveal any significant difference ($p > 0.05$) between the treatments. The average dry biomass value was estimated at 1.91±0.64 g/0.5l. This result shows that the dose of *Musa paradisiaca* crude extract does not significantly influence biomass production. These results are lower than the observations of Mutlen *et al.* (2019) who obtained an 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. In fact, *Musa paradisiaca* peels crude extract was used to prepare the semi-organic culture media in our experiments, whereas *Laportea aestuans* and *Manihot esculenta* crude extracts were used by these authors to prepare the experimental culture substrates. This difference in terms of culture substrate could explain the difference in growth of *Arthrospira platensis* in these media.

A comparative analysis of the soluble protein content of the post-harvest biomass from the different experimental growing media based on *Musa paradisiaca* peels crude extract compared with Jourdan's reference medium showed no significant difference ($P = 0.74 > 0.05$) between the treatments. This result shows that the dose of *Musa paradisica* peels crude extract does not influence the soluble protein content of the post-harvest biomass from the different experimental media. These results are higher than those obtained by Young-In (2012), i.e. a maximum average value of 43%/g of biomass. This difference could be justified by the difference in experimental culture media, in particular Jourdan's reference medium and media based on *Musa paradisiaca* peels crude extract (in the context of our experiments); Zarrouck's reference medium and media based on waste water enriched with CO2 (for this author), which could have a differential effect on the extraction of soluble proteins.

An analysis of the general pigments in the post-harvest biomass from the different experimental culture media revealed a similar effect of the different treatments on the chlorophyll A and B content ($p > 0.05$), i.e. respective mean values of 28.53±2.37 µg/g (for chlorophyll A) and 16.58±7.93 µg/g (for chlorophyll B). This result shows that the extract dose has no influence on the chlorophyll content of the post-harvest biomass. The chlorophyll levels obtained are nevertheless lower than those reported by Manet (2016), whose average was estimated at 60 mg/10g. This low content could be associated with the time of harvest, which would influence the availability of this pigment in the samples. The results reveal a variation in the content of these pigments depending on the harvesting period. However, analysis of carotenoid and phycocyanin content revealed a significant difference between treatments ($p= 0.008<0.05$; $p= 0.014<0.05$). These results indicate a significant effect of the culture medium on carotenoid and phycocyanin content. Analysis of the correlation between harvesting period and carotenoid and phycocyanin content revealed a positive and significant correlation between these parameters ($R2=0.68$). This result shows that the harvesting period has an influence on carotenoid and phycocyanin content. The average phycocyanin values obtained are lower than those recommended by Falquet (2006), i.e. content greater than 15 mg/g. The same applies to carotenoids, for which the average values obtained remain below those reported by Manet (2016), i.e. values of between 15-24

mg/10g. This low content of these constituents in the post-harvest biomass could be associated with the harvesting period and the drying technique, which could alter the constituents present in the harvested biomass.

A quantitative analysis of some secondary metabolites (alkaloids, flavonoids, total phenols, tannins) of post-harvest biomasses from experimental growing media shows a variation in the content of these compounds during the experimental phase. However, statistical analysis did not reveal any significant difference between treatments in terms of alkaloids ($P=0.20 > 0.05$), tannins ($p = 0.33 > 0.05$) and total phenols ($p = 0.47 > 0.05$). These results show that the culture medium does not influence the availability of alkaloids, total phenols and tannins in the biomasses. The average alkaloid value obtained is higher than that obtained by Lahocine (2019), i.e. an average value of 0.156 mg/ml. However, the total phenol content was higher than that obtained by Abd El-Baky et al (2009), with a maximum value of 16.96 mg/g. This observable difference could be associated with the difference in culture media, which would influence the total phenol content of the post-harvest biomass. Above all, it could be linked to the difference in spirulina strains used in the work (*Arthrospira platensis* in our experiments and *Spirulina maxima* in the work of these authors). However, analysis of the flavonoid content of post-harvest biomass revealed a significant difference between treatments ($P=0.009 < 0.05$). These results indicate a significant effect of the growing medium on the flavonoid content of the post-harvest biomass. Similarly, an analysis of the correlation between harvest time and flavonoid content revealed a positive and significant correlation between these parameters ($R2=0.68$). These results are higher than those obtained by Abd El-Baky *et al*. (2009), with an average value of 5.12mg/g. This difference could be associated with the different culture substrates.

V. Conclusions

The results of this study highlighted the mineral potential of *Musa paradisiaca* peels crude extract and their potential use as an input in the preparation of new growing substrates for *Arthrospira platensis*. Analysis of some of the major mineral constituents of *Musa paradisiaca* peels extract revealed a higher proportion of magnesium and calcium, with mean values of 390.333 ± 0.44 mg/l and 224 ± 0.66 mg/l respectively. On the other hand, the proportions of potassium and sodium were relatively low, with mean concentrations of 14.03 ± 0.04 mg/l and 17.36 ± 0.23 mg/l respectively. These results show that the dose of *Musa paradisiaca* crude extract does not significantly influence biomass production. The same applies to the soluble protein content of post-harvest biomass. An analysis of the general pigments in the post-harvest biomass from the different experimental environments revealed a significant effect of the growing medium and the harvesting period on the carotenoid and phycocyanine content. However, the dose of extract did not influence the chlorophyll (A and B) content of the post-harvest biomass. Quantitative analysis of some secondary metabolites (alkaloids, flavonoids, total phenols, tannins) in post-harvest biomass from experimental culture media showed a significant effect of experimental media on flavonoid content in post-harvest biomass. However, these results show that the growing medium does not influence the availability of alkaloids, total phenols and tannins in the biomass.

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Conflicts of interest

The authors declare no conflicts of interest.

References

[1]. Abd El-Baky, Hanna H., El Baz, Farouk K., El –Baroty, Gamal S., 2009. Production Of Phenolic Compounds From Spirulina Maxima Microalgae And Its Protective Effects In Vitro Toward Hepatotoxicity Model. Electronic Journal Of Environmental, Agricultural & Food Chemestry. Snn:1579-4377,Vol 8, Issue 12, P1099.

[2]. Aoac.(2000). Analysis Of The Association Of Official Analytical Chemists. (Ed. William, H.), 17ed., Gaithersburg, Md, Usa, Pp.141-144.

[3]. Arvouet-Grand A, Vennat B, Pourrat A, Legret P. Standardisation D'un Extrait De Propolis Et Identification Des Principaux Constituants. Journal De Pharmacie De Belgique. 1994;49:462-468.

[4]. Assemand, E., Camera, F., Kouame, F., Konan, V. And Kouame, L.P. (2012) Biochemical Characterization Of Plantain (Musa Paradisiaca) Variety 'Agnrin' Fruits In Côte D'ivoire And Sensory Evaluation Of Its Derivatives. Journal Of Applied Biosciences, 60: 4438-444.

[5]. Bate-Smith Ec, Swain T. New Leuco-Anthocyannins In Grasses. Nature. 1967;213:1033-1034.

[6]. Benahmed-Djilali A., 2012. ″Analyse Des Aptitudes Technologiques De Poudres De Dattes (Phoenix Dactylifera. L) Améliorées Par La Spiruline. Etudes Des Propriétés Rhéologiques, Nutritionnelles Et Antibactériennes″. Doctorat D'etat En Génie Des Procédés, Université M'hamed Bougara, Boumerdes, Algérie. 180p.

[7]. Bennett A, Bogorad L. 1973 Complementary Chromatic Adaptation In A Filamentous Blue-Green Alga. J Cell Biol 58(2): 419–435.

[8]. Boussahel S., Dahamna S., Ruberto G., Siracusa L., Harzallah D.,(2013). Phytochemical Study And Antioxidant Activities Of Leaves Extract From Rhamnus Alaternus L. Pharmacognosy Communications. Bangalore Vol 3 No 1 (Jan-Mars 2013): 46-53.

[9]. Doumandji A. , Boutekrabt L. , Saidi Nabil A., Doumandji S. , Hamerouch D. Et Haouari S. (2012).Etude De L'impact De L'incorporation De La Spiruline Sur Les Propriétés Nutritionnelles, Technologiques Et Organoleptiques Du Couscous Artisanal . Revue « Nature & Technologie ». N° 06/Janvier 2012. Pp 40 -50.

[10]. Durand-Chastel H.(1980).″Production And Use Of Spirulina In Mexico″,In: Algae Biomass. Shele, G. And Soeder J.(Edits), Elsevier, North Holland, Biomedical, Amsterdam. Pp. 51–64. 1980.

[11]. Fairchild,C.D&Glazer,A.N.(1994).Nonenzymatic Bilin Addition To The A Subunit Of Anapophycoerythrin. Journal Of Biological Chemistry, 269:28988-28996. Https://Pdfs.Semanticscholar.Org/7f94/ F4e07496786ee9bccbe5cd9ac82f0f90e524.Pdf.

[12]. Falquet J., 2006. ″Spiruline, Aspects Nutritionnels″. Antenna Technologie, Genève.22p.

[13]. Habib, B.; Parvin, M.; Huntington, C. And Hasan, R. (2008). A Review On Culture, Production And Use Of Spirulina As Food For Humans And Feeds For Domestic Animals And Fish. Fao Fisheries And Aquaculture Circular No. 1034, P. 33.

[14]. Hamadou Hh, Kallo Ms, Manzo Lm, Moussa I, Adamou R, Khalid I. Criblage Phytochimique Et Dosage Des Polyphenols Du Detarium Microcarpum Guill. Et Perr. Utilisé Dans Le Traitement Des Maladies Parasitaires Au Niger. Afrique Science. 2018;14(5):390-399.

[15]. Happi Emaga T, Bindelle J, Agneesens R, Buldgen A, Wathelet B, Paquot M. Ripening Influences Banana And Plantain Peels Composition And Energy Content. Trop. Anim. Health Prod. 2011;43(1):171177.

[16]. Egbuonu, Anthony And Nneji, Wisdom And Ukasoanya, Chimnonye (2017) Comparative Evaluation Of Some Nutrient Contents And Antifungal Properties Of Ground Musa Paradisiaca (Plantain) Peels And Leaves. British Journal Of Applied Science & Technology, 19 (1). Pp. 1-8. Issn 22310843. Https://Doi.Org/10.9734/Bjast/2017/28393

[17]. Egbuonu Acc, Ogele Om, Amaraihu Kl. Comparative Evaluation Of The Proximate Composition And Antibacterial Activity Of Ground Musa Paradisiaca (Plantain) Peels And Leaves. British Biotechnology Journal. 2016;15(2):1-9. Doi: 10.9734/Bbj/2016/2715.

[18]. James Cj. The Analytical Chemistry Of Food. Chapman And Hall New York, 2005.

[19]. Jourdan. J.P. (1996). "Sugar As A Source Of Carbon For Spirulina (Arthrospira Platensis) Culture". International Symposium On Cyanobacterial Biotechnology", Bharathidasan University, Tiruchirapalli, Inde.

[20]. Jourdan J.P.(1999),″Cultivez Votre Spiruline: Manuel De Culture Artisanale″.Genéve, Pp85 ;63.

[21]. Kouadio Kb, Koffi Ma, Oussou Kr, Ahoua Rc, Kablan Alc, Dongui Bk, Et Al., Phytochemical Screening, Determination Of Total Polyphenols And Flavonoids, And Evaluation Of The Antibacterial Activity Of Leaves Of Turraea Heterophylla Smith (Meliaceae). Journal Of Pharmacognosy And Phytochemistry. 2021;10(5):16-21.

[22]. Lahoucine Halimz A. (2019). Etude De L'impact De L'incorporation De La Spiruline Sur La Qualité Organoleptique Et Physico-Chimique De La Mayonnaise. Mémoire De Master En Agronomie, Spécialité Biotechnologie Alimentaire. Département D'agronomie Faculté Des Sciences De La Nature Et De La Vie ; Université Abdelhamid Ibn Badis-Mostaganem (Algérie). 80p.

[23]. Mahamane Iia, Bakasso S, Hama Gr, Hama Hh, Hassane Hz, Ilagouma At, Et Al. Phenolics Contents And Antioxidant Activity Of Six Medicinal Plants Used In The Treatment Of Dentition Related Ailments In Niger. Journal Of Pharmacongosy And Phytochemistry. 2020;9(4):1816- 1821.

[24]. Manet A. (2016) : La Spiruline : Indications Thérapeutiques, Risques Sanitaires Et Conseils A L'officine. Sciences Pharmaceutiques. 2016. Ffdumas-01346709f.116p.

[25]. Miliauskas G, Venskutonis Pr, Van Beek Ta. Screening Of Radical Scavenging Activity Of Some Medicinal And Aromatic Plant Extracts. Food Chem. 2004;85:231-237.

[26]. Mutlen M., Fokom R., Tomedi E. M., Dibong S. D., Simeni Y. H., And Boyomo S. B. S. (2019). Contribution A L'étude D'une Cyanophycée : Effet Comparé Des Extraits Des Feuilles De Manihot Esculenta Crantz (Euphorbiaceae) Et De Laportea Aestuans (Urticaceae) Sur La Croissance Et La Qualité De Arthrospira Platensis En Culture Artisanale Dans La Localité De Yabassi-Cameroun. International Journal Of Innovation And Scientific Research. Issn 2351-8014 Vol. 43 No. 1 Jun. 2019, Pp. 25-40.

[27]. Nafack Tsazeu Judicaël Boris, Ngo Nkot Laurette, And Kana Sop Marie Modestine, "Plantain (Musa Paradisiaca L.): Production, Consumption And Processing In Cameroon." American Journal Of Food Science And Technology, Vol. 11, No. 1 (2023): 8-14. Doi: 10.12691/Ajfst-11-1-2.

[28]. Onwuka C.F.I., Adetiloye P.O., Afolami C.A., 1997. Use Of Household Wastes And Crop Residues In Small Ruminant Feeding In Nigeria. Small Rumin. Res.;24:233237.

[29]. Oyeyinka, B.O.; Afolayan, A.J., 2019.Comparative Evaluation Of The Nutritive, Mineral, And Antinutritive Composition Of Musa Sinensis L. (Banana) And Musa Paradisiaca L. (Plantain) Fruit Compartments. Plants 2019, 8, 598. Https://Doi.Org/10.3390/Plants8120598.

[30]. Patricia Amenan Kouadio, Barthélemy Koffi Attioua, Faustin Aka Kabran, Brise Amian Kassi, Rosmonde Affoué Yao, Herman Kouamé Yeboue, Marcel N'dri Kasse, Ernest Kouakou Amoikon (2020). Comparative Physico-Chemical Study Of Fermented And Unfermented Cocoa Beans From Côte D'ivoire. International Journal Of Biosciences 16 (1), (2020): 355-362. Issn: 2222-5234,2020.

[31]. Rangel-Yagui, O.; Danesi, G.; Carvalho, M. And Sato, S.(2004). ″Chlorophyll Production From Spirulina Platensis: Cultivation With Urea Addition By Fed-Batch Process″. Bioresour. Technol., 92(2):133–141.

[32]. Ribereau-Gayon P, Stonestreet E. Annales De Chimie. 1966;48:188-196.

[33]. Richmond, A.(1988). ″Spirulina″, In: Microalgal Biotechnology (Eds. Borowitzka, A. And Borowitzka, J.), Cambridge University Press, Cambridge. Pp. 85-121.

[34]. Sguera Sébastien (2008). Spirulina Platensis Et Ses Constituants: Intérêts Nutritionnels Et Activités Thérapeutiques. Sciences Pharmaceutiques. Hal-01732214. 176p.

[35]. Singleton Vl, Orthofer R, Lamuela-Raventos Rm. Analysis Of Total Phenols And Oxidation Substrates And Antioxidants By Means Of Folin-Ciocalteu Reagent. Methods In Enzymo. 1999;299:152-178.

[36]. Sreevidya, Narasimhan And Mehrotra, S. (2003). Spectrophotometric Method For Estimation Of Alkaloids Precipitable With Dragendorff″S Reagent In Plant Materials. J. Aoac 2003; 86, 1124–1127.

[37]. Strosse H., Schoofs H., Pams B., André E., Reymiers K., Swennen R., 2006. Development Of Embryonic Cell Suspensions From Shoot Meristematic Tissue In Bananas And Plantains (Musa Spp.). Plant Sci. 2006; 170: 104-112.

[38]. Theodore, S. And Georgios, S. (2013). Health Aspects Of Spirulina (Arthrospira) Microalga Food Supplement.J. Serb. Chem. Soc., 78 (3): 395-405.

[39]. Vonshak, A. & Borowitzka, M.A., 1991.Laboratory Manual: Research Seminar And Workshop On Mass Cultures Of Microalgae. Silpakorn University, Thailand. 40pp.
[40]. Vonshak, A., 1997. Spirulina Platensis (Arthrospira): Physiology, Cell Biology And Biotechnology. Taylor And Francis, London 1997.Isbn 0 748406743. 233pp.,Gbp 49.95.
[41]. Vonshak, A.; Chanawongse, L.; Bunnag, B. And Tanticharoen, M. (1996).″Role Of Light And Photosynthesis On Acclimation Process Of Cyanobacterium Spirulina Platensis To Salinity Stress″. J. Appl. Phycol., 8:119-124.
[42]. Vonshak, A, (2002). ″Use Of Spirulina Biomass″, In: Spirulina Platensis (Arthrospira) Physiology Filament Biology And Biotechnology. (Ed. Vonshak, A.), Taylor & Francis, Isbn, London, Pp. 159-173.
[43]. Warburg & Christian W, (1941). Isolierung-Und Kristallisation Des Garungsferments Enolase. Biochem.Z., 310,384-42.
[44]. Young-In Park (2012).Bioconversion Du Co2 En Biomasse Algale Et Utilisation De L'eau Usée Municipale Comme Source Additionnelle De Carbone Et D'oligoéléments. Mémoire De Maîtrise Spécialité : Environnement ; Département De Génie Chimique Et Génie Biotechnologique. Faculté De Génie. Université De Sherbrooke (Canada).91p.
[45]. Zarrouk C. (1999). Contribution A L'étude D'une Cyanophycée : Influence De Divers Facteurs Physiques Et Chimiques Sur La Croissance Et La Photosynthèse De Spirulina Maxima (Setch Et Gardner) Geitler. Thèse Doctorat, Faculté Des Sciences, Université De Paris.83pp.