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CHEMICAL COMPOSITION OF *SPIRULINA PLATENSIS* OF NOMAYOS-YAOUNDE (CAMEROON)

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ABSTRACT
Background*: S. platenis is rich in macronutrients and micronutrients. It is used as a dietary supplement and found in tropical and semi-tropical areas This study aims to investigate the chemical composition of S. platensis of Nomayos (Yaounde, Cameroon) focusing on macro and micronutrients and phyotchemical bioactive molecules*
Materiel and methods*: S. platensis was collected in a farm in Nomayos (Yaounde, Cameroon). The aqueous extract was used for the determination of nutrients using standard methods. Trace elements were determined by atomic absorption spectrophotometry. The phytochemical composition was determined by using HPLC. Phycocyanin and Carotenoids were determined using chemicals methods.*
Results*: The extraction yield obatined was 16.84%. The results showed that S platensis contains protein (375.5±0.7 g/kg dw), lipids (301.2±11.9 g/kg dw), carbohydrates (243.9±9.9g/kg dw) and fibers (313.2 g/kg dw). The HPLC profile revealed the presence of polyphenols (21.2 ± 1.18 mg eq. QE/g Ext.), flavonoids (56.4 ± 6. 47 mg eq. QE/g Ext.) and phenolic acid like caffeic and coumaric acidss. Iron was the most micronutrients found but we also found copper, manganese, zinc selenium. The percentage of phycocyanin was 16.15% while carotenoids were 3.8%.*
Conclusion*: S. platensis from Nomayos have significant concentration of nutrients. However other studies need to be carried out in order to determine the nature of different monomers present in protein, lipids, carbohydrates and other macronutrients found in this microalgae.*

Keys words : spirulina, chemical composition, phycocyanin, polyphenols.

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1. Introduction

Spirulina platensis (Oscillatoriaceae) is a blue-green microalgae that has been found by several scientists and nutritionists to be a functional food with exceptional nutritional qualities (FAO, 1991). The cultivation of *S. platensis* is widespread all over the world with high productivity and low cost of production (Charpy , Langlade, & Alliod , 2008). This algae preferably grows in warm alkalinated waters rich in nitrogen and phosphorus nutrients such as in some of the lakes in Africa, latin America, and South Asia. In addition it is found in tropical and semi-tropical areas (Castenholz & al., 2001). The thermophilic character of *S. platensis* and its important needs of light limit its range to a tropical belt between approximately 35° north and 35 ° south latitude. *S. platensis'* production takes place at several levels: artisanal, semi-industrial and industrial. The differentiating factors of these production methods are the total area of cultivation tanks, the equipment, and technology used in the production process (IRD, 2008). As mentioned above, *S. platensis* is present in various part of the world, and depending on its location, it takes on a different name. For instance in the far North region of Cameroon it is called sembe''; in Chad it is called ''dihe''. Rich in proteins, minerals, trace elements, and many vitamins such as B1, B2, B12 and E, this microalgae is considered an unconventional food resource (Sall & al.,1999). In Cameroon it is produced and used as a functional food, however no data has been published regarding its composition. Therefore our study aims to investigate the micro and macronutrients composition of *S. platensis* produced at Nomayos in Cameroon.

2. Material and methods

Spirulina platensis was provided from a farm situated at Nomayos (Yaounde- Cameroon). The algae was cultivated inside a basic water tank with pH 10. Each day a third of the culture medium was collected on a fabric filter which passed water while retaining the spirulina accumulated on the very fine mesh filter (30 to 40µ). This spirulina was then pressed for 20 minutes to obtain a biomass which was spread out on racks in the form of spaghetti. Next it would be dried in an electric dryer at 40 °C, a temperature that will ensure a better preservation of vitamins and phytonutrients. The dried algae was then grinded into a fine powder.

2.1. Preparation of the aqueous extract

The aqueous extract was obtained by macerating 100 g of *S. platensis* powder with 1000 mL of distilled water. This mixture was then placed on intermittent agitation for 24 hours, and the filtrate obtained was immediately lyophilized.

2.2. Determination of macronutrients and micronutrients

The determination of total ash protein, carbohydrates, lipids, and fibers were determined using standard methods (Van Soest, 1967; AOAC, 1980; AOAC, 1984; Bergeret, 1985). The determination of micronutrients was carried out using atomic absorption spectrophotometry after extraction in a mixture of nitric- hydrochloric acid (75v/ 25v).

2.3. Phytochimical screening and phenolic profile of S.platensis

2.3.1. Determination of the phenolic content of the extract

The total phenol, flavonoids, and flavonols contents were determined by standard methods (Singleton & Rossi, 1965; Zhishen & al., 1999; Kumaran & Karunakaran, 2006).

2.3.2.Total antioxidant activity by Ferric Reducing Antioxidant Power assay (FRAP)

The Total antioxidant activity was determined using documented method by Benzie and Strain (Benzie & Strain, 1996) with some modifications (Moukette & al., 2015). The fresh solution of FRAP reagent consisted of acetate buffer (300 mM; pH 3.6), 2,4,6- Tri (2-pyridyl)-s-triazin (TPTZ) (10 mM), and FeCl3•6HO (50 mM) in the ration 10v:1v:1v. For the assay 75 µL of S. platensis extract and 2 ml of FRAP reagent were mixed for 12 minutes. The absorbance of this mixture was measured at 593 nm. . The vitamin C was used to draw a standard curve and the results were expressed as mg equivalent vitamin C/ g of dried extract (mg eq VitC/g DE).

2.3.3.Quantification of phenolic compounds by HPLC

High Performance Liquid Chromatography (HPLC) with UV detection was used for identification and detection of phenolic profile of the extract. The solution of S. platensis extract was dissolved in pure water in the ratio of 30 mg extract/1mL water and centrifuged at 4706 rpm for 10 minutes. The supernatant was filtered through a cellulose acetate membrane filter 0.20 µm - 0.45µm, (Schleicher & Schuell). The analysis of the filtrated solution was performed on an Agilent Technologies 1200 HPLC system fitted with a SUPELCOSIL LC-18 column (length 250 mm, diameter 4.6 mm, packaging size 5 mm). The column temperature was set at 20°C. The mobile phase was made of a mixture of solution of acetic acid 0.5% by volume (A) and acetic nitrile (B). The elution was performed by using 100% of A for the first 2 minutes of the run, 40% of A and 60% of B from 2 to 60 minutes. The flow rate was set equal to 1 mL/min and the volume 25 µL was injected in the column. Polyphenols were detected by a UV detector (280 nm). The retention times of the identified different phenolic compounds were measured using a single standard solution (100 mg/L). This method was also previously used by Moukette and al (Moukette & al., 2015).

2.4.Determination of phycocyanin and carotenoids

For the dermination of phycocyanin, 3g of *S. platensis* was diluted with 100mL of distilled water and decanted. Then 20mL of supernatant were centrifuged (3000 rpm, 10 minutes) and 0.5 mL of the supernatant was diluted 100 times with distilled water. The optical density (OD) of each sample was

spectrophotometrically measured at 615 and 652 nm (Jourdan, 2006).

Regarding the investigation of carotenoids concentration in the *S. platensis* extract, 3 g of *S. platensis* powder were extracted with 25 mL of acetone; the mixture was kept for 24h in the fridge. The surpernatant was centrifuged at 3000 rpm for 10 minutes and 0.5 mL of it was diluted 100 times. The OD was read at 450 nm (Vonshak & Borowitzka, 1991).

3. Results And Discussion

The extraction yield obatined was 16.84% and was calculated using the following formula: Rd = Mass of the extract obtained × 100 / initial powder mass. The percentage of humidity was 07.24%+0.26. The nutritional composition of *S. platensis* presented in table 1 showed the presence of high level of protein and iron. *S. platensis* of Nomayos demonstrated a lower concentration of protein and some trace elements compared to the one harvested in Switzerland (65% of protein), Burkina Faso (61.3 % of protein) and Chad (58.61% and 50.24% of protein) (Azabji & al., 2011; Branger & al., 2003; Ngakou & al., 2012). The differences could be explained by either the growth environment, the difference in climate, or the techniques used to collect S. platensis (Clement, 1975). The protein content in this algae could vary with the sampling period in relation to the photoperiod with the highest contents being obtained at the beginning of the sunlight period (AFAA, 1982). *S. platensis* of Nomayos showed more lipids than the one harverted in Chad (Ngakou & al., 2012). This difference could be due to a variation of the extraction method or the type of solvent used. Furthermore our study revealed that the level of iron was 25.6 mg/100g DM. This is higher compared to that found in other studies (0.7 to 1.2 mg/100g DM) (Montasell, 2009).

The phytochemical screening revealed the presence of polyphenols (56.4 + 6.47 mg eq. QE/g Ext.), total flavonoids (21.2 + 1.18 mg eq. QE/g Ext.), total flavonols (13.2 + 0.6 mg eq. QE/g Ext.); and total antioxidant capacity (7.5 + 0.33 mg eq. VitC/g Ext.). The phenolic profile of *S. platensis* using high performance liquid chromatography showed the presence of several phenolic acids especially caffeic, coumaric O and P, gallic, and other bioactive molecules at different levels (Table 2). Our results are similar to other studies which demonstrated the presence of phenolic and other bioactive molecules like phycocyanin (Bhavisha & Parula, 2010). We obtained 16.15% of phycocyanin which is more than the 15% obtained by Fox (1986) and Jourdan (1999). The carotenoid pigments including ß-carotene are important as the main source of vitamin A represented 3.80%.

Table 1: Nutritional composition of *S. platensis* of Nomayos -Yaounde

Macro-nutrients (%)	**Dry weight**	**Lipids**	**Proteins**	**Fibers**	**Sugars**	**Energy**
	92,76±0.26	30.12±1.19	37.55±0.07	31.32±7.95	24.39±0.99	518.84 kcal
Micro-nutrients (mg /Kg)	**Iron**	**Manganese**	**Copper**	**Zinc**	**Selenium**	**Ash**
	256.56± 0.01	23.38±0.00	28.95±0.00	25.01±0.01	1.24±0.01	07.93±0.20

Table 2: HPLC polyphenols profile of *S. platensis*

Polyphenol standard	**Standard Retention time**	***Spirulina platensis***	
	TR(min)	Con [mg/L]	A(mAU) (mg/g DW)
Gallic	14.38	**422.87**	$1.65x10^4$
3,4-OH benzoic	19.10	41.92	1062.86
Tyrosol	21.76	40.91	700.29
OH-Tyrosol	21.906	70.15	759.48
Catechine	23.48	66.84	919.68
O-Coumaric	25.10	**140.87**	4409.89
Caffeic	25.66	**518.63**	$1.68x10^4$
P-Coumaric	30.51	**201.68**	1.03e4
Apigenin	33.49	1.66	7333.82

Conc: concentration; DW: dried weight; T.R.: retention time; *A*: area

The diversity of micro and macronutrients found in *S. platensis* increased the focus on this algae and its used as a marketed food supplement in many countries in Europe, Africa, Asia and India. Studies demonstrated important properties of *S. platensis* such as an antioxidant, antiviral, antibacterial, antidiabetic and other biological activities (Lee & al., 2001; Bhavisha & Parula, 2010). Recent studies also demonstrated benefit effects of S. plantensis from Nomayos on patients living with HIV/AID by increasing the level of CD4 cells count, reducing the viral loads and increasing HDL levels (Ngo Matip & al., 2014; Ngo Matip & al., 2015). The benefits from *S. platensis* could be attributed to either its micronutrients or to other bioactive molecules in the algae.

4. Conclusion

Given its chemical composition and richness in micro and macronutrients, as well as other bioactive molecules, we can attest to the benefits of *S. platensis* from Nomayos. However other studies need to be carried out in order to determine the nature of different monomers present in protein, lipids, carbohydrates and other macronutrients found in this microalgae.

Author's contribution: VJAM and CAP conducted the study; PCNB, BMM, TNF and PN did the analysis and carried the study, MENM gave *S. platensis* and JN supervised the study. All the authors approve the content of the work.

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References

[1] **AFAA** (French Association for Applied Algology). *First symposium on Spirulina platensis Gom. Geitleir of AFAA* 1982.

[2] **AOAC**. *Official methods of analysis of the association of official analytical chemists*(13rd ed) William Horturitz ed. Washingtoon DC. 1980.

[3] **AOAC**. *Official methods of analysis association of official analytical chemists.* Centennial edition 1884 – 1984 (14 th ed), Virginia 22209 USA. 1984

[4] **Azabji, K. M., Loni, G.E., Sobngwi, E., Onana, E. A., Edie, D.S., Denis von der Weid, & al.** The Effect of Spirulina platensis versus Soybean on Insulin Resistance in HIV-Infected Patients: A Randomized Pilot Study. Nutrients 2011; **3** :712-724

[5] **Benzie, I.F.F. & Strain, J.J (1996).** Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Analytical Biochemistry 1996 ; **239**:70-76

[6] **Bergeret, B.** La chimie analytique dans un laboratoire d'outre-mer. Organisation des techniques de laboratoire : étude critique des techniques d'analyses employées au laboratoire de la section nutrition de l'IRCAM – ORSTOM, IRCAM. 56p 1955.

[7] **Bhavisha, R. & Parula, P.** Spirulina : Potential clinical therapeutic application. Journal of Pharmacy Research 2101 ; **3**(8) :1726-1732

[8] **Branger, B., Cadudal, J.L., Delobel, M., Ouoba, H., Yameogo, P., Ouedraogo, D., Guerin, D., Valea ,A., Zombre, C. & Ancel, P.; personnels des CREN** (2003). Spiruline as a food supplement in case of infant malnutrition in Burkina-Faso. Archives de Pediatrie 2003 ; **10**(5):424-31

[9] **Castenholz, R.W., Rippka, R., Herdman, M. & Wilmotte, A.** Form-genus I. Arthrospira 2001.

[10] **Clement, G**. Production et constituants caractéristiques des algues *Spirulina platensis et maxima*. Annales de la Nutrition et de l' Alimentation 1975 ; **29** : 447-488.

[11] **Charpy, L., Langlade, M.J. & Alliod, R.** « La Spiruline peut-elle être un atout pour la santé et le développement en Afrique ? » ; IRD : 67 P. 2008.

[12] **Cornet, J.F.**Cinétic and energetic study of aphoto-bioreactor. Doctorate Thesis, University de Paris-South Orsay Centre. In: for de spirulina production. Jourdan, J.P (Ed.). Antenna Technology, 146pp. 1992.

[13] **FAO/WHO/UNU**. Protein Quality Evaluation. Report of the Joint FAO/WHO ExpertConsultation: 1985 FAO/WHO/UNU 2- to 5-yearold requirement pattern. FAO; 51. 1991.

[14] **Fox , R.D.** Algoculture : la spiruline, un espoir pour le monde de la faim. Edisud, Aix-en-Provence, ISBN 2-85744-262-9, 319 p.1986.

[15] **Jourdan, J.P.** Grow your spirulina. In: Jourdan, J.P. (Ed). Antenna Technology. 146pp. 2006.

[16] **Kumaran, A. & Karunakaran, R.J.** Nitric oxide radical scavenging active components from Phyllanthus emblica L. Plant Foods for Human Nutrition 2006 ; **61**(1):1-5.

[17] **Lee, J.B., Srisomporn, P., Hayashi, K.,Tanaka, T., Sankawa, U. & Hayashi, T.** Effects of Structural Modification of Calcium Spirulan, a Sulfated Polysaccharide from Spirulina Platensis, on Antiviral Activity. Chemical and Pharmaceutical Bulletin 2001 ; **49**(1) : 108—110

[18] **Mitchell, G.V., Grundel, E., Jenkins, M. & Blakely, S.R.** Effects of graded dietary levels of Spirulina maxima on vitamins A and E in male rats. Journal of Nutrition, 1990 ; **120**(10): 1235-40
[19] **Montasell.** Montasell-Import SARL, Spirulina,Pressbook, p. 12-13. 2009.
[20] **Moukette Moukette, B., Pieme, C.A., Nya Biapa, P.C., Njimou, J.R. & Ngogang Yonkeu**, J. Free radicals quenching potential, protective properties against oxidative mediated ion toxicity and HPLC phenolic profile of a Cameroonian spice: Piper guineensis. Toxicology Reports 2015 ; **2**:792–805.
[21] **Ngakou, A., Ridine, W., Mbaïguinam, M. & Namba, F.** Changes in the physico-chemical properties of Spirulina platensis from three production sites in Chad. Journal of Animal & Plant Sciences 2012 ; **13**(3): 1811-1822
[22] **Ngo-Matip, M.E., Pieme, A.C., Azabji-Kenfack, M., Nya Biapa, P.C., Nkenfack, G., Heike, E., Moukette Moukette, B., Korosky, E.l., Mbofung, C.M. & Yonkeu Ngogang, J.** Effects of Spirulina platensis supplementation on lipid profile in HIV–infected antiretroviral naïve patients in Yaounde - Cameroon: a randomized trial study. Lipids in Health and Disease 2014 ; **13**:191
[23] **Ngo-Matip, M.E., Pieme, C.A., Azabji-Kenfack, M., Moukette Moukette, M., Korosky, E., Stefanini, P., Yonkeu Ngogang, J. & Mbofung, C.M.** Impact of daily supplementation of Spirulina platensis on the immune system of naïve HIV-1 patients in Cameroon: a 12-months single blind, randomized, multicenter trial. Nutrition Journal 2015 ; **14**:70
[24] **Pinero Estrada, J.E., Bermejo Besco´, P. & Villar del Fresno, A.M.** Antioxidant activity of different fractions of Spirulina platensis protean extract. Il Farmaco 2001 ; **56** :497–500
[25] **Sall, M.G., Dankoko , B., Badiane, M., Ehua, E. & Kuakuwi, N.** Résultats d'un essai de réhabilitation nutritionnelle avec la Spiruline à Dakar. Médecine d'Afrique Noire 1999 ; **46**:143-14
[26] **Singleton, V. & Rossi, J.** Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture 1965 ; **16**:144–58.
[27] **Stizenberger**. Bergey's Manual of Systematic Bacteriology (D. R. Boone & R.W.
[28] Castenholz, eds.) 1852 ; **1**: 542-54
[29] **Van Soest, R.J. & Wine, R.H.** JAOAC (50) Washington DC. 1967.
[30] **Vonshak, A. & Borowitzka, M.A.** Laboratory Manual: Research Seminar and Workshop on Mass Cultures of Microalgae. Silpakorn University, Thailand. 40pp. 1991.
[31] **Zhishen, J., Mengcheng, T. & Jianming, W**. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide. Radicals Food Chemistry 1999 ; **64**:555–9.