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Growth And Chemical Components Of Spirulina Subsauce Grown With Agricultural Fertilizers At Different Nitrogen Concentrations

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Abstract

The article discusses cyanobacteria of the genus Spirulina, in particular Spirulina subsalsa, and their cultivation in low-cost media using agricultural fertilizers as a source of nutrients. It is mentioned that Spirulins are widely used in various biotechnological applications due to their high content of proteins, vitamins, minerals and antioxidant pigments.

It is noted that the morphological characteristics of Spirulinas may vary due to factors such as cultivation conditions, which may lead to errors in their taxonomic identification. Despite this, its potential in medical applications is highlighted, such as in the treatment of cancer, hyperlipidemia, kidney and liver diseases, diabetes and neurodegenerative diseases.

The study describes the isolation and identification of Spirulina subsalsa from water samples from the Clavellinos reservoir, Venezuela, as well as its subsequent cultivation in media enriched with agricultural fertilizers at different nitrogen concentrations. Parameters such as population growth, biochemical composition, and pigment content of cultured cyanobacteria are evaluated.

The results show that Spirulina subsauce grown in low-cost agricultural environments has similar nutritional values to those obtained with the conventional Zarrouk medium, suggesting its potential use in the biotechnology industry and in aquaculture as feed.

Key words: *Spirulina, crop, biotechnology, nutrients.*

INTRODUCTION

Cyanobacteria or blue-green algae comprise a heterogeneous group of photoautotrophic prokaryotes (VAN-D[1]EN-HOEK ET AL., 1995) with broad biotechnological application (ENCARNAÇÃO et al., 2015; SOMMELLA et al., 2018). Within this group, the genus Spirulina stands out, often confused with Arthrospira, although it is now fully clarified that they correspond to two totally different genera (ALI & SALEH, 2012). A series of characteristics are used for its classification, such as shape, size of the filaments, shape of their trichomes and coils, among others; however, these characteristics may vary due to morphological changes induced by factors such as cultivation conditions as well as phenotypic plasticity, sometimes

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leading to errors in the taxonomic identification of strains (LYRA et al., 2001; VALERIO et al., 2009).

Species of the genus Spirulina grow in alkaline media and possess spiral or helical filaments, and are widely used as a source of proteins, vitamins, and minerals, such as anti-cancer drugs (FEDOROV et al., 2013; OUHTIT ET AL., 2014), HYPERLIPIDEMIA (Deng & CHOW, 2010), kidney and liver damage (GALLARDO et al., 2011; RODRÍGUEZ ET AL., 2012), DIABETES (Layam & KASI, 2006) and different neurodegenerative diseases (SALVADOR et al., 2011).

Likewise, Spirulina possesses a variety of pigments: carotenoids, xanthophylls, chlorophyll, and phycobiliproteins (SAINI et al., 2018), which are influenced by temperature (Lee ET AL., 2012; PANDEY & TIWARY, 2010; USLU et al., 2011), light intensity (Lee ET AL., 2012; PANDEY & TIWARY, 2010; RAVELONANDRO et al., 2011), nitrogen concentrations (Colla ET AL., 2007a; COLLA et al., 2007b; USLU et al., 2011), among others.

Similarly, microalgae of the genus Spirulina are high-protein sources, rich in vitamins, essential amino acids, minerals, essential acids (gamma-linolenic acid), and antioxidant pigments such as carotenoids (BELAY et al., 1997, SORIANO, 2017). In addition to having a high nutritional value, they have immunomodulatory function and affectivity proven to protect against radiation (TAKEUCHI et al., 2002).

Spirulina cultures are usually performed in fresh water and with expensive culture media due to the inclusion of a large number of analytical-grade salts. These media include the Zarrouk (ZARROUK, 1966), Spirulina (AIBA & Ogawa, 1977), BG-11 (Rippka, 1988) AND SOME MODIFIED MEDIA (Amala & Ramanathan, 2013; KUMARI et al., 2014a; KUMARI et al., 2014b). This situation has led to the search for alternative sources of crop media that include the use of agricultural fertilizers, in order to make it possible to obtain high yields of biomass at low cost.

In this vein, KUMARI et al. (2014A), COMPARED A MEDIUM ENRICHED WITH NPK 10:26:26 AND Zorrouk medium, for the culture of S. platensis, finding excellent results with respect to growth rate, nitrogen fixation rate, and protein and lipid accumulation. AMALA & RAMANATHAN (2013) compared the growth and biochemical composition of S. platensis grown in a low-cost medium, called RME, formulated with NaNO3 and K2HPO4, and Zarrouk medium, reporting that there were no differences in biomass content, chlorophyll contents, and protein and lipid contents, in Spirulina strains grown with both media. SOPANDI et al. (2020), worked with S. platensis with goat manure as a culture medium showing in their results a higher growth of S. platensis of 2.425±0.097g/L at a concentration of 75 g/L. The protein content in S. platensis was greater than 75 (62.56 ± 6.04%) g/L, however, lipids and carbohydrates in S. platensis presented the highest contents at the concentration of 25 g/L (7.86 ± 1.16%-27.69 ± 1.78%).

In addition to nutrients, there are other environmental factors that influence the growth and chemical composition of Spirulina, such as: temperature, pH, and lighting, among others (COUTEAU, 1996). Knowledge of the physiological characteristics and specific requirements of each microalgal strain is of utmost importance, as it would maximize its biotechnological potential.

In this regard, LN.CITLALLI (2021) found that temperatures and lighting influence the growth and biochemical components of Arthrospira maxima, showing that the maximum biomass value was 1.07 g/L, while the percentage of proteins were 40.42± 7.9%, carbohydrates 51.86% and lipids of 6.1 ± 0.25%, when cultured with a Light intensity of 150 µmol photons/m2·s and with photoperiod of light 12 h of light: 12 h of darkness and temperature of 25°C. On the other hand, MATEUCCI (2018) worked with Arthrospira (Spirulina) plantis LMPA55", at a temperature of 26 ± 2°C and a light intensity of 34 µE.m-2.s-1, reporting maximum values of final biomass 2.10 ± 0.02 $^{g\ PS/L}$ and proteins 22%.

Taking into consideration the above approaches and related to the economic importance of Spirulina, the evaluation of the growth, chemical components and pigment

content of Spirulina subsauce grown with agricultural fertilizers at different nitrogen concentrations was scheduled, which would be a novel report for the biotechnology of cyanobacteria isolated from ecosystems in Venezuela.

MATERIALS AND METHOD

Isolation and Identification of Cyanobacteria Spirulina Subsauce

Based on the studies of BERNAL (2002) who pointed out the presence of Spirulina in the Clavellinos reservoir, Sucre state, Venezuela, a sampling was scheduled in the aforementioned area. Water samples were taken superficially (0-5 m deep) with sterile plastic collectors with a capacity of 350 mL, between 0-5 m away from the shore of the reservoir (coordinates: between 10° 19' to 10° 23' Lat. N and between 63° 35' to 63° 40' Long. O), and later taken to the Microalgae Biotechnology Laboratory of the Department of Fisheries Biology of the Oceanographic Institute of Venezuela, at the Universidad de Oriente.

In the laboratory, reservoir water samples were diluted in previously sterilized distilled water (120 ºC, 15 psi, 15 min). Subsequently, the samples were inoculated with the help of a seeding loop in petri dishes containing 15 mL of Spirulina solid medium (AIBA & OGAWA, 1977) and incubated at a temperature of 25±1ºC, illumination of 39 µE/m2s and photoperiod 12:12, until the appearance of blue-green colonies. The latter were taken with a seeding loop and resuspended in liquid Spirulina medium for purification. Once obtained, the pure colonies of cyanobacteria were placed in sterile glass tubes with 15 mL capacity Bakelite lids, containing 10 mL of Spirulina liquid medium and kept in a culture chamber under the aforementioned environmental conditions.

When growth was evidenced, the purity of the strain was verified through microscopic observations at 40X and it was safeguarded for subsequent identification, which was carried out in the Phytoplankton Taxonomy Laboratory of the Department of Biology of the Universidad de Oriente, Venezuela, using morphological criteria and following the taxonomic keys proposed by AGUIAR (2013). The isolated strain was incorporated into the Algae Germplasm Bank of the Oceanographic Institute of Venezuela, Universidad de Oriente, with the code BGAUDO 161.

Growth and chemical components of Spirulina subsauce grown with agricultural fertilizers at different nitrogen concentrations.

For the preparation of the culture medium, filtered and ozonated tap water was used, which was enriched with the T20BS culture medium (because it only contains in its composition: Triple 20 agricultural fertilizer (20:20:20), sodium bicarbonate (4 g/L) and sodium chloride (1 g/L) and four concentrations of nitrogen (7.5, 15, 30 and 60 mmol/L) were evaluated. The inocula for these cultures came from previously acclimatized cultures.

The selection of nitrogen concentrations was based on those used by the Zarrouk medium (ZARROUK, 1966), which is the most widely used in the cultivation of Spirulina and has a nitrogen concentration of 29 mmol/L. Using this concentration as a reference, it was decided to evaluate the influence of higher (2X) and lower (X/2 and X/4) nitrogen concentrations on the growth and accumulation of pigments in S. subsalsa.

Cultures were carried out intermittently, in triplicate, under controlled environmental conditions (T: 30±1 ºC; illumination: 3000 lux; manual agitation, photoperiod 12:12), for 15 days. Glass jars containing 3 L of culture medium were used (Figure 2). The initial population density was 0.5±0.025 mg/L, and from the beginning of the trial and every 72 h, samples of

each of the treatments were taken to determine pH and population growth according to the criteria of PELIZER & OLIVEIRA (2014).

Figure 2.- Culture of Spirulina subsauce for 15 days in glass jars, containing 3 L of culture medium at different concentrations of nitrogen.

When the crops reached the exponential phase, the entire crop was harvested, filtered in a permaline bag. The harvested biomass, after several washes with acidulated water (pH 4), was placed in a stove at 50ºC until a constant mass was obtained. Subsequently, this biomass was kept refrigerated at 6ºC until the time of biochemical composition analyses, which included total proteins (LOWRY et al., (1951); carbohydrates (DUBOIS et al., 1956); lipids (extraction according to BLIGH & DYER, 1959 and quantification by PANDE et al., 1963), chlorophyll a (JEFFREY & HUMPHREY (1975) and carotenoids (STRICKLAND & PARSONS, 1972).

After the respective analyses, the nitrogen content that led to the highest growth and protein content of Spirulina was selected for six-fold cultivation under identical conditions. The biomass was harvested as noted above; after drying, it was pulverized in a mortar and the flour obtained was reserved for use as feed in the culture trials of the rotifer Brachionus plicatilis and the postlarvae of the white shrimp Littopeneus vannamei.

Chemical Constituents

Total proteins (LOWRY et al., 1951):

The method of LOWRY et al. (1951) combines the Biuret reaction with the reduction of the Folin-Ciocalteu reagent (phosphomolybdic and phosphotungstic acids) by the oxidation of tyrosine, tryptophan, cysteine of polypeptide chains. The oxide-reduction process is accompanied by the formation of a characteristic blue color.

5–10 mg of Spirulina subsauce biomass was placed in Falcon-type test tubes with 5 mL of 1 eq/L NaOH, then taken to a water bath (95–100 °C) for 1 hour. They were then centrifuged, and 50 and 100 µL of this extract were taken in triplicate, to complete with NaOH 1 eq/L up to a volume of 1 mL, 1 mL of a 50:1 solution of Na2CO3 at 2% with NaOH 0.1 eq/L: CuSO4.5H2O at 0.5% in sodium tartrate and potassium at 1%, Shake and let sit for 10 minutes. After this time, 100 µl of a 1:1 solution of Folin-Ciocalteu reagent: H2O was added, stirred and left to rest in darkness for 30 minutes, and the absorbances were read at 750 nm (A750) in a Jenway spectrophotometer, model 6405. Previously, a standard curve of seroalbumin solution (BSA) was made, from which the value of the slope (a) and intersection with the y-axis (b) was obtained, to later calculate the concentration of total proteins (mg/mL, mg/g and % m/m), using the following equations:

$$\text{Conc. proteína en el extracto (mg/mL extracto)} = \left[\frac{A750-b}{a}\right] \times \left[\frac{Vt(\mu L)}{Vm(\mu L)}\right]$$

$$\text{Conc. proteína (mg/g de biomasa)} = \text{conc.prot.en el extracto} \times \left[\frac{Vf(mL)}{masa(g)biomasa}\right]$$

$$\text{Conc.de proteína (\%)} = \text{Conc. de proteínas(mg/g de biomasa)} \times 0{,}1$$

where:

Vt = total volume of the reaction.

Vm = sample volume.

Vf = final volume of the extract.

Total Lipids

a) Extraction (Bligh & Dyer, 1959)

5-10 mg of Spirulina subsauce biomass was placed in Falcon-type test tubes and 5 mL of chloroform: methanol (1:2 V/V) plus 100 µL of a BHT solution in chloroform (0.1%) were added. They were carefully shaken with a glass rod until the biomass was broken and the tubes were protected with aluminum foil, and they were protected at 4ºC, for 24 hours. After this time, the samples were centrifuged at 3000 r.p.m. for 10 minutes. The supernatant was collected and transferred to a clean tube. A second extraction was performed with 3 ml of chloroform-methanol mixture (2:1 V/V). 2 mL of distilled H2O was added to the supernatant, stirred with vortex until a homogeneous and dense solution was obtained, and then centrifuged at 3000 r.p.m. for 10 minutes. The aqueous upper stage was removed with a Pasteur pipette and discarded. The organic phase was transferred to a glass tube and 0.5 mL of acetone was added to the chloroform phase to help remove traces of water. The chloroform phase evaporated at a temperature of 37-40 ºC for 24 hours in a stove. The tubes were stored at -20°C until the quantification of the total lipids by spectrophotometry.

b) Quantification (Pande et al., 1963):

To the concentrate that remained in the glass tubes (lipids) after the evaporation of chloroform, 3 ml of the 2% acid dichromate solution was added to each tube (first to the whites). The tubes were covered with aluminum foil, and placed in a water bath at 100ºC, for 15 minutes; Subsequently, they were allowed to cool to room temperature (15 minutes). Once cooled, 4.5 mL of distilled water was added. These were vigorously mixed in vortex and allowed to cool again (15 minutes), and then read the absorbance on a Jenway spectrophotometer, model 6405, at 590 nm (A590). Previously, a standard curve was made with tripalmitin in chloroform, from which the value of the slope (a) and intersection with the y-axis (b) were obtained, to subsequently calculate the concentration of total lipids (mg/mL, mg/g and % m/m), using the following equations:

$$\text{Conc. de lípidos (mg/g de biomasa)} = \frac{\left[\frac{A590-b}{a}\right]}{\text{masa (g)de biomasa}}$$

Conc. lipid (%) = Lipids (mg/g biomass) x 0.1

Carbohydrates (DUBOIS et al., 1956):

This method proposed by DUBOIS et al. (1956) is based on the fact that carbohydrates are particularly sensitive to strong acids and high temperatures. Under these conditions a series of complex reactions take place starting with simple dehydration, if heating and acid catalysis are continued, several derivatives of furan are produced that condense with themselves and with other by-products to produce colorful compounds, products of the condensation of phenolic compounds and with heterocycles with nitrogen as a heteroatom. The most common condensation is with phenol. This method is easy, effective, and fast.

5–10 mg of Spirulina subsauce biomass was placed in Falcon-type test tubes with 5 mL of 1 eq/L NaOH; then, they were taken to a water bath (95-100°C) for 1 hour. They were then centrifuged, and 50 and 100 µl of this extract were taken in triplicate, to complete with NaOH 1 eq/L up to a volume of 1 mL. Then, 0.5 ml of 5% phenol and 2.5 mL of concentrated sulfuric acid were added, stirred and placed at 100 ºC for 15 minutes. After this time, they were placed in an ice bath, left to rest for 15 minutes, until they reached room temperature and then read the absorbances at 490nm in a Jenway spectrophotometer, model 6405.

Previously, a standard curve was made with anhydrous glucose (12 mg of glucose in 100 mL NaOH 1 eq/L), from which the value of the slope (a) and intersection with the y-axis (b) was obtained, and then the concentration of carbohydrates (mg/ml, mg/g and % m/m) was calculated, using the following equations:

$$\text{Conc. carboh. en el extracto (mg/ml)} = \left[\frac{A490-b}{a}\right] \times \left[\frac{Vt(\mu l)}{Vm(\mu l)}\right]$$

$$\text{Conc. de carboh. biomasa (mg/g)} = \text{conc.prot.en el extracto} \times \left[\frac{Vf(mL)}{masa(g)biomasa}\right]$$

Conc.de carbohidratos (%)= Conc. de carboh.(mg/g biomass) x 0.1

where:

Vt = total volume of the reaction.

Vm = sample volume.

Vf = final volume of the extract.

Photosynthetic pigments (JEFFREY & HUMPHREY (1975). STRICKLAND & PARSONS (1972):

Chlorophyll A and Total Carotenoids

5-10 mg of biomass was placed in test tubes and 5 mL of acetone was added: methanol (2:1 v/v); They were then placed in the dark at 4°C for 24 h to ensure complete extraction of the pigments. They were then centrifuged at 3500 rpm for 5 min and the supernatant was absorbent at 480, 647 and 664 nm on a spectrophotometer (Jenway brand, model 6405 UV/V). The absorbances obtained were used for the quantification of chlorophyll a content according to the methodology of JEFFREY & HUMPHREY (1975):

Chlorophyll a (µg/mL) = 11.93 (A664) - 1.93 (A647)

Total carotenoids were determined by the equation proposed by Strickland & Parsons (1972):

Total carotenoids (μg/mL) = 4 (A480)

Statistical analysis of the results

Prior to compliance with the assumptions of homogeneity of variances and normality, the data on the values of biomass, biochemical components and pigments of S. subsalsa, obtained at the end of the trial, in each of the different treatments, were contrasted by means of an analysis of variance of one factor (nitrogen concentration), following the recommendations of SOKAL & ROLHF (1995).

RESULTS

Identification of the cyanobacterium Spirulina subsauce

The cyanobacterium isolated from the Clavellino reservoir is filamentous, cylindrical-helical with slightly rounded ends, with isolated trichomes, pale blue-green and homogeneous cell content. Their coils are narrow, juxtaposed, dense, and regular. The filaments have a diameter between 1-2.3 μm, with distance and height between the turns between 1-2 μm and 2-3.3 μm, respectively. No gas vesicles or calyptra were observed at the cell endings (Figure 3). These characteristics, together with those presented by AGUIAR (2013) AND Luo & Jiang (2015), ALLOW US TO CONCLUDE THAT THE CYANOBACTERIUM ISOLATED IS Spirulina subsalsa.

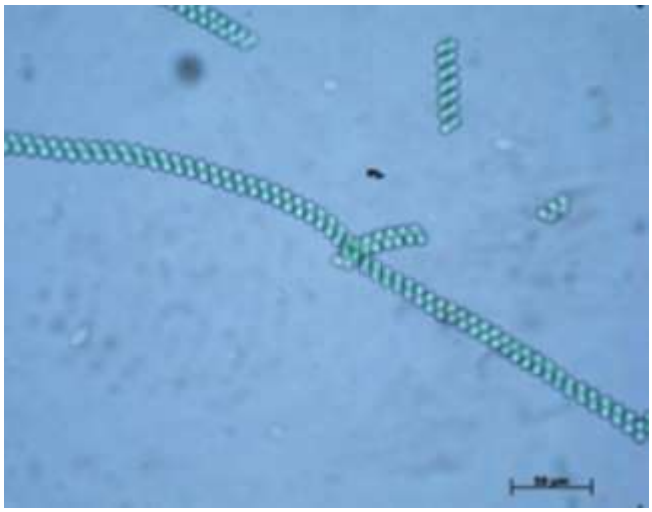

Figure 3. Micrograph of Spirulina subsalsa isolated from the Clavellino Reservoir, Sucre state, Venezuela. Source: ROMERO et al. (2018).

Growth, chemical constituents and pigment content of the Spirulina subsauce strain grown at different concentrations of nitrogen with agricultural fertilizers.

The growth of the S. subsalsa strain at different nitrogen concentrations is shown in Figure 4. At the end of the trial, significant differences were observed between the 30 mmol/L nitrogen treatment and the rest of the treatments ($P < 0.05$; Annex 1), with this concentration of 30 mmol/L causing the highest biomass production (2.63 mg/mL).

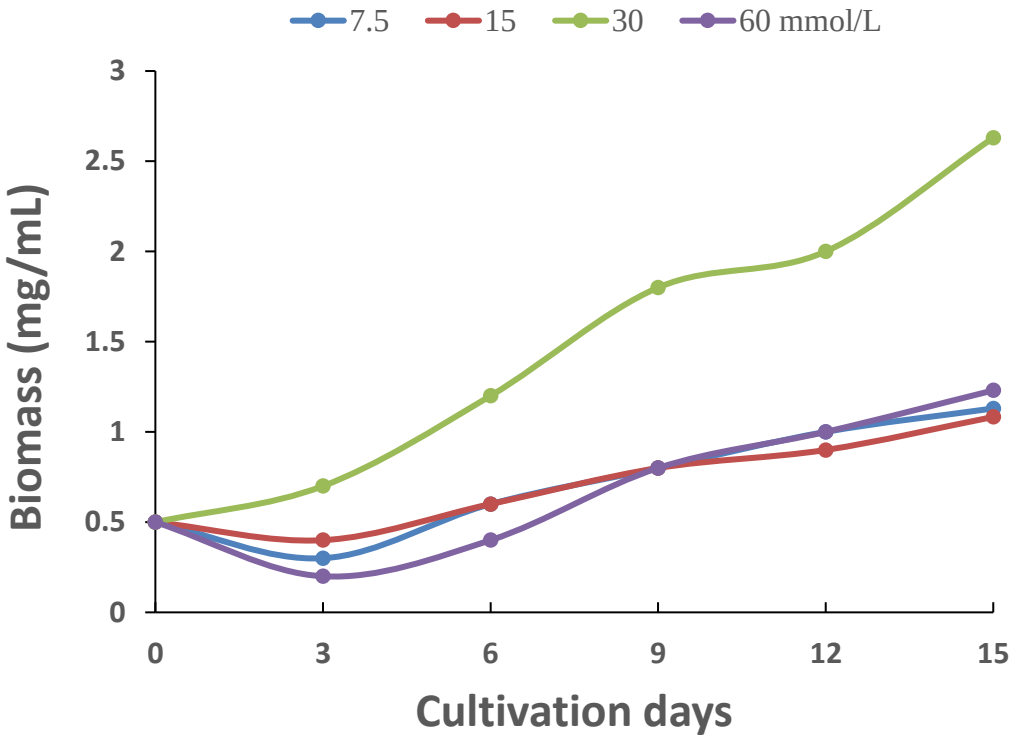

Figure 4. Growth of S. subsauce grown in an agricultural environment at different nitrogen concentrations (7.5, 15, 30 and 60 mmol/L).

Chemical Constituents

a) Total Protein, Total Carbohydrate, Total Lipids, and Pigments:

The total protein contents (Figure 5) showed significant differences ($P < 0.05$; annex 2) in the different treatments, with the concentration of 30 mmol/L of N causing the highest values of this macromolecule (45.86%).

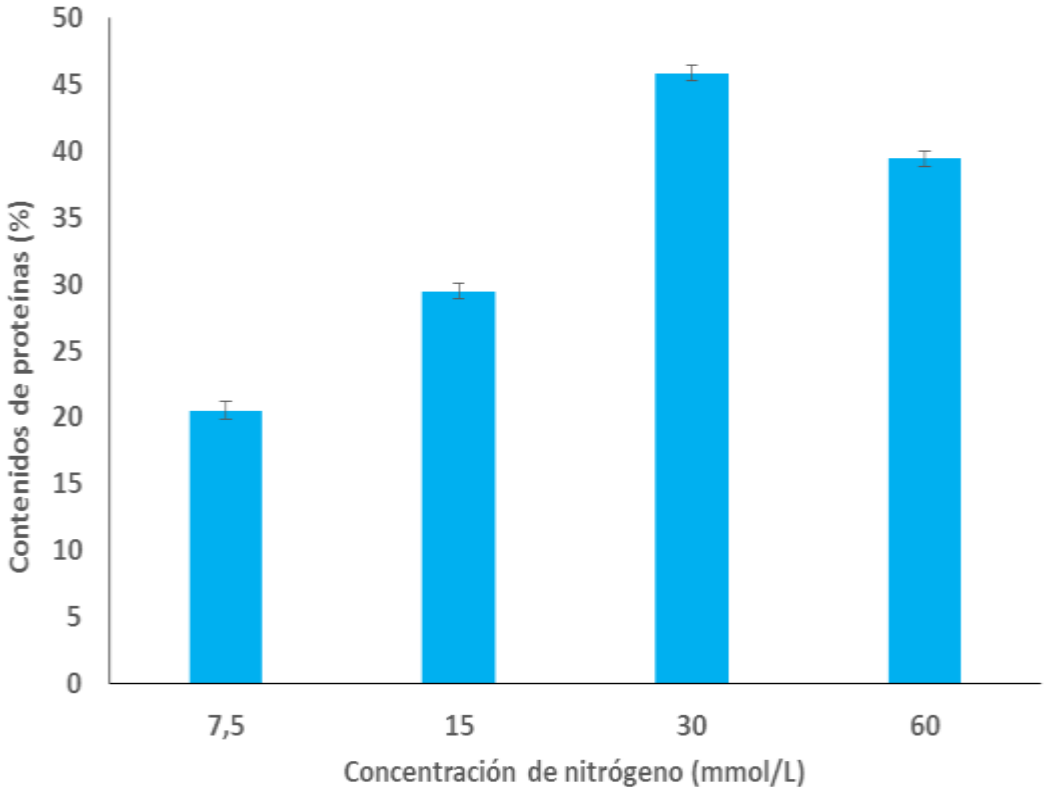

Figure 5. Total protein content (%) of Spirulina subsauce grown in an agricultural environment at different nitrogen concentrations (7.5, 15, 30 and 60 mmol/L). In original Spanish language

Total carbohydrates (Figure 6) showed significant differences ($P < 0.05$; Annex 3) in the different treatments, with the highest contents (30.16%) being observed in the nitrogen concentration of 7.5 mmol/L.

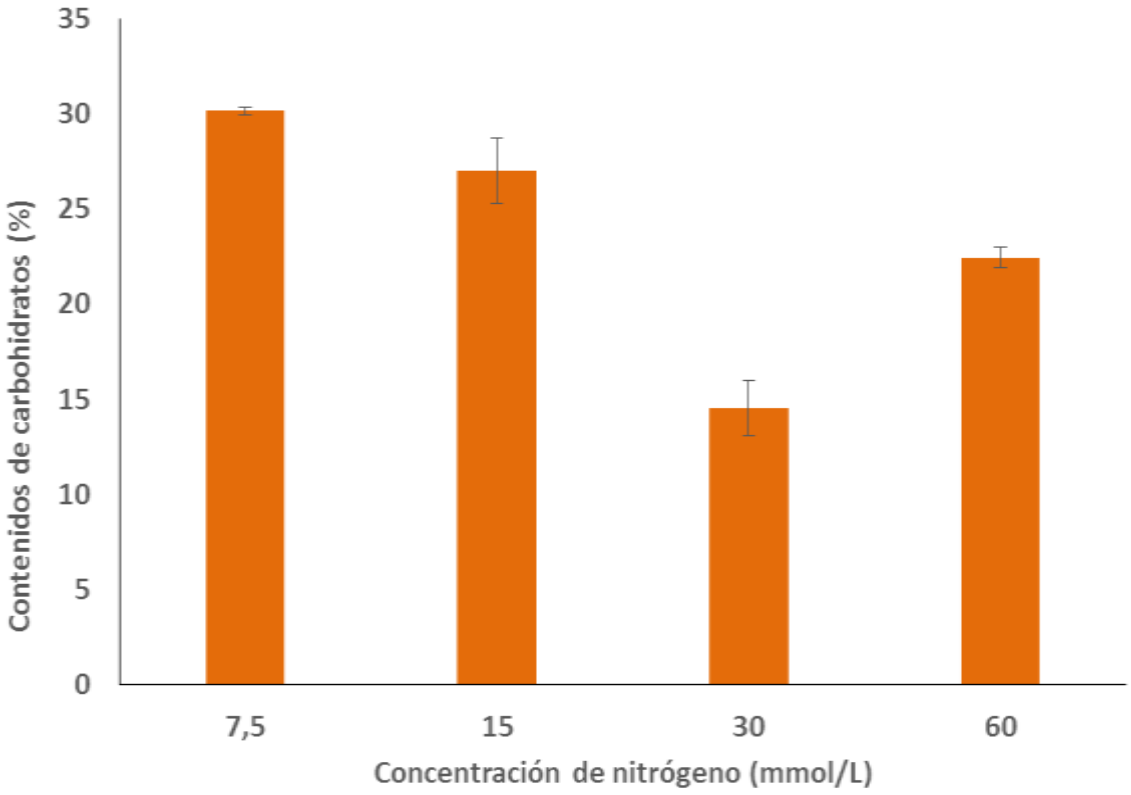

Figure 6. Carbohydrate content of Spirulina subsauce grown in an agricultural environment at different nitrogen concentrations (7.5, 15, 30 and 60 mmol/L). In original Spanish language

On the other hand, total lipids showed a similar behavior to carbohydrates, showing significant differences ($P< 0.05$; annex 4) in the different treatments and reaching the highest content (28.56%) in the nitrogen concentration of 7.5 mmol/L (Figure 7).

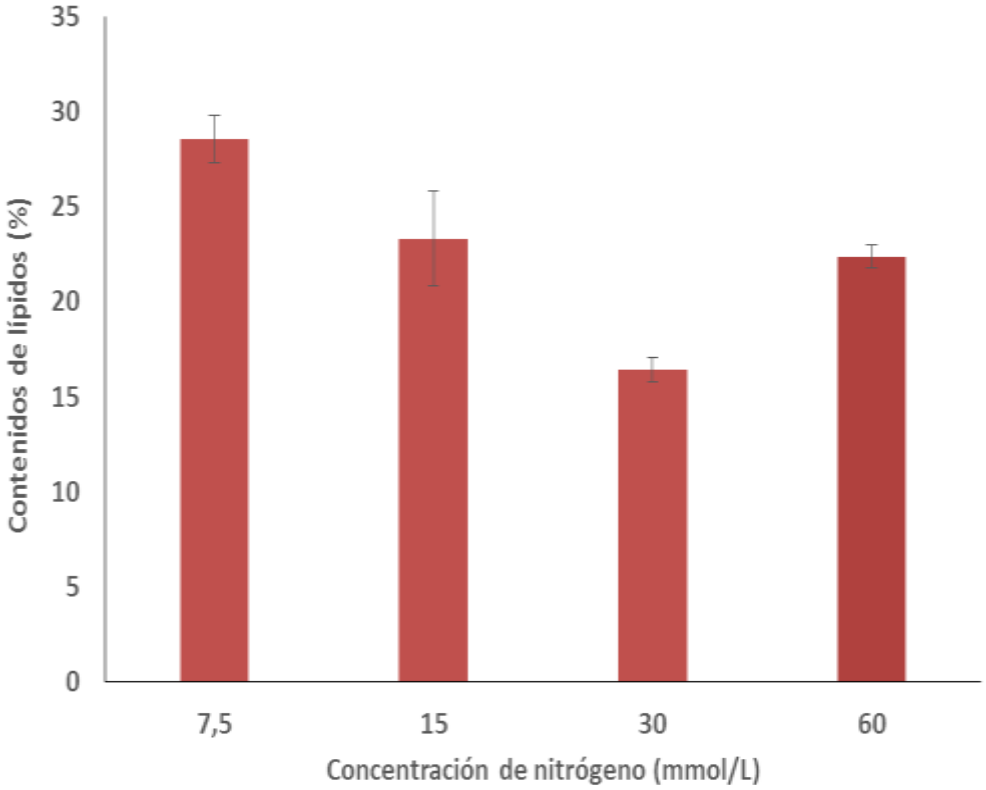

Figure 7. Total lipid content of Spirulina subsauce grown at different nitrogen concentrations (7.5, 15, 30 and 60 mmol/L). In original Spanish language

The contents of chlorophyll a and total carotenoids showed a similar trend, i.e., their contents decreased with increasing nitrogen supply in the crops (Figure 8). Both pigments showed significant differences ($P< 0.05$; Annex 5 for chlorophyll A and Annex 6 for total carotenoids) in the different treatments. The highest values of chlorophyll a and total carotenoids obtained were 6.42 µg/mL and 1.41 µg/mL in cultures with 7.5 mmol/L of nitrogen, respectively.

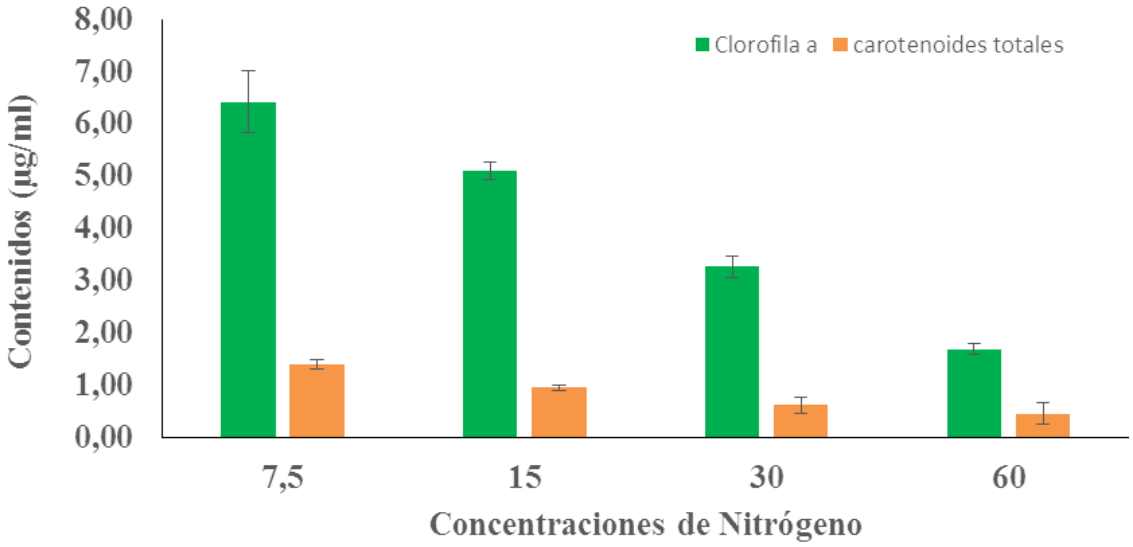

Figure 8**.** Chlorophyll a and total carotenoid contents of S. subsauce grown at different nitrogen concentrations (7.5, 15, 30 and 60 mmol/L). In original Spanish language

DISCUSSION

The identification of cyanobacteria as Spirulina subsalsa was made based on morphological characters; however, additional molecular studies are needed to confirm its correct identification, since it has been shown that the phenotypic plasticity of cyanobacteria causes them to acquire different morphologies when exposed to significant changes in environmental conditions, particularly temperature. nitrogen concentration and salinity, among others (ROMERO, 2019; LYRA et al., 2001; VALERIO et al., 2009).

In Venezuela, S. subsalsa and other members of the genus Spirulina have been observed in different environments, such as Lake Maracaibo (RODRÍGUEZ, 2001), the Pao-Cachinche reservoirs (boundaries between the states of Carabobo and Cojedes), La Mariposa (capital district), La Pereza and Quebrada Seca (Miranda state), El Lagartijo (Miranda state) and in the Los Roques Archipelago (GONZÁLEZ et al., 2003; PETRASH et al., 2012); however, there is only one report on its cultivation in the country (ROMERO et al., 2018).

Studies of microagal biomass production in alternative culture media have increased over the years, as production costs with commercial media are not competitive for the market. In this regard, VIEIRA et al. (2001) studying the use of different concentrations (10 – 30 – 50 mM) of commercial grade nitrogen sources (ammonium chloride, ammonium acid phosphate, ammonium nitrate, urea and ammonium sulfate), obtained biomass values of 0.993 g/L with 10 mM ammonium nitrate and 0.910 g/L with 10 mM urea. AVILA-LEON et al. (2012) using urea at different concentrations (0.5 – 5 mM) in semi-continuous cultures with different dilution rates (0.04 – 0.44 d-1) obtained 1.415 g/L to 5 mM of urea, values that are lower than those obtained in this research (2.63 mg/mL = 2.63 g/L), a result that is probably due to the lower concentration of nitrogen used by the previous authors and variations in culture conditions.

However, by including other factors in Spirulina crops, higher biomass contents can be achieved, such as, for example, MARREZ et al. (2013) evaluated the effect of various culture media on the biomass and pigment content of Spirulina platensis, finding in the medium Zarrouk (NO3 concentration of 29 mmol/L) greater amount of biomass (4.87 g/L) than that obtained in this work. On the other hand, JIAN et al., 2015, worked with Spirulina subsauce obtaining a maximum biomass production (2.86 mg L1) when it was grown in Zarrouk medium supplemented with wastewater (CW: monosodium glutamate), at 25%.

The highest total protein content (46%) of S. subsalsa, grown with agricultural fertilizer in this research, was obtained when 30 mmol/L of nitrogen was used (fig. 5), surpassing that published by NARANJO (2019) who obtained 39.4% in Arthospira sp. crops with 10 mM NH4NO3; 41%) reported by JIAN et al. (2015), when they grew Spirulina subsauce in 25% wastewater supplemented (CW) Zarrouk medium. These results lead us to suppose that low ammonium values induce a greater accumulation of proteins in Spirulina, which may be due to the fact that ammonium is directly assimilated and directed to the synthesis of amino acids, indicating that this element is more energetically favorable for cells (ABALDE et al., 1995).

Total carbohydrates in cyanobacteria have a variable behavior and will depend not only on the particular species, but also on the interaction of the physicochemical factors established in the crops, since they are the energy components that organisms in general use most easily and quickly for their metabolism ALVAREZ (2018). Thus, it has been determined that variations in culture conditions can increase protein production in Spirulina, as reported by ROMERO (2019) who, when cultivating S. subsalsa at 9 UPS and 14 mM of nitrogen, obtained the highest contents of this macromolecule in the exponential phase (58.5%).

In this study, carbohydrates had the highest values (30.16%) in the nitrogen concentration of 7.5 mmol/L (Fig. 6); differing from MATEUCCI (2018) who worked with Arthrospira (Spirulina) plansis LMPA55", at temperatures of 26 ± 2°C and a light intensity of 34 μE.m-2.s-1, obtaining carbohydrate contents of 8.8 % PS at a nitrogen concentration of 3.75 g/L. Similarly, these values are higher than those determined by JIAN et al. (2015), authors who worked with Spirulina subsauce grown in modified Zarrouk medium supplemented with wastewater (CW) by 50%, obtaining a maximum carbohydrate content of 18%. However, they contrast with the values found by NARANJO et al. (2019) who found the highest carbohydrate content in Arthospira sp (228.69 ± 0.01 mg/g; 22.87%). when they used 10 mM of NH2CONH2 as a source of nitrogen. On the other hand, ROMERO (2019) when cultivating S. subsalsa at 14 mM of nitrogen determined 17.1± 0.91% in carbohydrate content. The behavior of the content of this macromolecule could be due to the use of low concentrations of sodium nitrate, which not only represent a decrease in cost, but also an increase in carbohydrate productivity.

Regarding total lipids, the highest contents (28.56%) were obtained at the nitrogen concentration of 7.5 mmol/L (Fig. 7), and it can be assumed that this nitrogen concentration favored these total lipid contents, as shown by LORETO et al. (2003), who indicated that microalgae cultures exposed to low nitrogen content generally experience a marked increase in lipid synthesis. It should be noted that the values obtained in this study were higher than those of MATEUCCI, (2018) WHO SUBJECTED Arthrospira (Spirulina) plantesis LMPA55", to a nitrogen concentration of 0.63 g/L, obtaining lipid contents of 19.2 % PS, and equally higher than those of Romero (2019) who, when cultivating S. subsalsa at 14 mM Lipid content of 14.1± 0.20% was determined. This could be due to the growing conditions and mainly to the low concentrations of nitrogen where the lipids reached the maximum contents.

In relation to pigment production, chlorophyll a and total carotenoids showed a similar trend to those observed in carbohydrates and lipids. The highest values of chlorophyll a and total carotenoids (6.42 μg/mL and 1.41 μg/mL, respectively) were obtained in cultures with 7.5 mmol/L of nitrogen (Fig. 8). Thus, it has been shown that nutrient sources, in particular nitrogen concentration, have a marked effect on the synthesis of chlorophyll a and carotenoids,

and it has been observed that the synthesis of these pigments decreases as the concentration of this macronutrient in crops decreases (BEN-AMOTZ & AVRON 1983b; PETER et al., 2010). Likewise, the accumulation of pigments in microalgae is correlated with light intensity, as well as with the photoperiod, finding that the optimal condition for obtaining higher chlorophyll contents in Spirulina sp. cultures can be in a photoperiod of 16 hours of light and 8 hours of darkness, and that continuous illumination tends to suppress the synthesis of this pigment. since it tends to photooxidize the photosynthetic apparatus (PAREEK & SRIVASTAVA, 2001).

Considering the culture conditions, ÁLVAREZ (2018) reported that Spirulina platensis LMPA55c when cultured at a photoperiod of 12:12, at a light intensity of 2800 lux and in Zarrouk medium with (29 mmol/L of N), the microalgae presented chlorophyll a contents of 1.08 ± 0.07%, likewise, values have been found in Arthrospira (Spirulina) plantesis LMPA55", when subjected to 26 ± 2°C, a light intensity of 34 μE.m-2.s-1 and a nitrogen concentration of 3.73 g/L, obtaining chlorophyll contents of 1.29% PS ([Mateucci], 2018); values that were lower than those referred to in this study (6.42 ug/mL). However, the values of the present study contrast with those of ROMERO et al., (2019) who, when cultivating S. subsalsa at 14 mM of nitrogen, reported chlorophyll a contents of 42.7±0.40 μg/mL and carotenoids of 157.9 ±2,36 μg/mL. The discrepancies observed in these results could be due to differences in the content of nitrogen concentrations and the growing conditions used in such investigations.

Regarding the physicochemical parameters that occurred during the development of the assays of this study, the pH of these varied between 9.20-10.00, which is within the range indicated for the optimal growth of this type of cyanobacteria (SOUNDARANDIAN & VASANTHI, 2010; RINCÓN et al., (2013).

The results obtained on the growth, contents of proteins, lipids, carbohydrates and pigments in the native strain of Spirulina subsalsa, when cultivated with agricultural fertilizer, allow us to suggest the feasibility of massively producing this cyanobacterium with a culture medium of lower cost than the one used conventionally (Zarrouk), lacking the evaluation of this biomass as feed for animals of aquaculture interest. as will be addressed in the following chapters.

CONCLUSIONS

The results obtained on the growth, biochemical components and pigments in the native strain of Spirulina subsalsa, grown in a low-cost agricultural environment, showed nutritional values similar to those reported with the Zarrouk medium, as they have been shown by other authors, which allow us to suggest the use of this cyanobacterium in the biotechnological industries with a view to its use as feed in aquaculture.

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ANNEXES

Annex 1. Statistical analyses of the biomass values of Spirulina subsauce obtained at different nitrogen concentrations.

Analysis of Variance

Fountain	Sum of Square	Mexico City	Half Squares	F-Rank	P-Value
Between groups	4,9934	3	1,66447	32,29	0,0001
Within Groups	0,4124	8	0,05155		
Total (Corr.)	5,4058	11			

Multi-Range Testing for Biomass by Concentration

Method= 95.0 Concentration	Percentages Counted	Tukey HSD Stocking	Groups Homogeneous
15 mmol/L	3	1,08333	X
7.5 mmol/L	3	1,13	X
60 mmol/L	3	1,23333	X
30 mmol/L	3	2,63333	X

Annex 2. Statistical analysis of the total protein values of Spirulina subsauce obtained at different nitrogen concentrations.

Analysis of Variance

Fountain	Sum of Square	Mexico City	Half Squares	F-Rank	P-Value
Between groups	1118,78	3	372,926	1026,17	0,0000
Within Groups	2,90733	8	0,363417		
Total (Corr.)	1121,69	11			

Multi-Range Testing for Proteins by Concentration

Method= 95.0 Concentration	Percentages Counted	Tukey HSD Stocking	Groups Homogeneous
7.5 mmol/L	3	20,52	X
15 mmol/L	3	29,47	X
60 mmol/L	3	39,4333	X
30 mmol/L	3	45,8867	X

Annex 3. Statistical analysis of the total carbohydrate values of Spirulina subsauce obtained at different nitrogen concentrations.

Analysis of Variance

Fountain	Sum of Square	Mexico City	Half Squares	F-Rank	P-Value

Between groups	413,677	3	137,892	103,03	0,0000
Within Groups	10,7071	8	1,33838		
Total (Corr.)	424,385	11			

Multi-Range Testing for Carbohydrates by Concentration

Method= 95.0 Concentration	Percentages Counted	Tukey HSD Stocking	Groups Homogeneous
30 mmol/L	3	14,5467	X
60 mmol/L	3	22,4467	X
15 mmol/L	3	26,9967	X
7.5 mmol/L	3	30,1633	X

Annex 4. Statistical analysis of the total lipid values of Spirulina subsauce obtained at different nitrogen concentrations.

Analysis of Variance

Fountain	Sum of Square	Mexico City	Half Squares	F-Rank	P-Value
Between groups	222,537	3	74,1789	34,07	0,0001
Within Groups	17,42	8	2,1775		
Total (Corr.)	239,957	11			

Multi-Range Testing for Lipids by Concentration

Method= 95.0 Concentration	Percentages Counted	Tukey HSD Stocking	Groups Homogeneous
30 mmol/L	3	16,4333	X
60 mmol/L	3	22,4	X
15 mmol/L	3	23,3333	X
7.5 mmol/L	3	28,5667	X

Annex 5. Statistical analysis of chlorophyll a values of Spirulina subsauce obtained at different nitrogen concentrations.

Analysis of Variance

Fountain	Sum of Square	Mexico City	Half Squares	F-Rank	P-Value
Between groups	38,5377	3	12,8459	118,97	0,0000
Within Groups	0,8638	8	0,107975		
Total (Corr.)	39,4015	11			

Multi-Range Testing for Chlorophyll A by Concentration

Method= 95.0 Concentration	Percentages Counted	Tukey HSD Stocking	Groups Homogeneous

60 mmol/L	3	1,7	X
30 mmol/L	3	3,26667	X
15 mmol/L	3	5,11333	X
7.5 mmol/L	3	6,41667	X

Annex 6. Statistical analysis of the total carotenoid values of Spirulina subsauce obtained at different nitrogen concentrations.

Analysis of Variance

Fountain	Sum of Square	Mexico City	Half Squares	F-Rank	P-Value
Between groups	1,54616	3	0,515386	26,62	0,0002
Within Groups	0,154867	8	0,0193583		
Total (Corr.)	1,70103	11			

Multi-Range Testing for Carotenoids by Concentration

Method= 95.0 Concentration	Percentages Counted	Tukey HSD Stocking	Groups Homogeneous
60 mmol/L	3	0,466667	X
30 mmol/L	3	0,633333	XX
15 mmol/L	3	0,963333	X
7.5 mmol/L	3	1,40667	X